Linking leaf initiation to the aerial environment

When air temperature is not the whole story

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To My Beloved Wife and Son

Contents

Abbreviations

DMC	Dry matter content
DW	Dry weight
e_{air}	Air vapour pressure
E_{bud}	Apical bud transpiration rate
e_{bud}	Saturation vapour pressure at bud surface
$E_{bud\ area}$	Apical bud transpiration rate per bud-contained leaf area
e_s	Saturation air vapour pressure
FLA	Final leaf area
FW	Fresh weight
LAR	Leaves appeared per unit of time (leaf appearance rate)
LED	Leaf expansion duration
LED_{dd}	Leaf expansion duration in thermal time
LER	Leaf expansion rate (mean)
LER_{dd}	Leaf expansion rate (mean) normalized for thermal time
LIR	Leaves initiated per unit of time (leaf initiation rate)
LIR_{DD}	Leaves initiated per unit of thermal time
LL	Leaf length
LUR	Leaves unfolded per unit of time (leaf unfolding rate)
LW	Leaf width (maximum)
PPFD	Photosynthetic photon flux density
RH	Relative humidity (%)
R_{LW}	Net absorbed longwave radiation (> 2800 nm)
R_{net}	Net radiation absorbed by a body (0-100 μm)
R_{SW}	Shortwave radiation (< 2800 nm)
SAM	Shoot apical meristem
SLA	Specific leaf area
T_{air}	Air temperature
T_{base}	Base temperature
T_{bud}	Shoot apical bud temperature
$T_{ceiling}$	Temperature of the glass ceiling of the climate room
T_{leaf}	Leaf temperature
$T_{meristem}$	Shoot apical meristem temperature
T_{plant}	Plant temperature
U	Wind speed
VPD	Vapour pressure deficit
$VPD_{bud\text{-}air}$	Vapour pressure difference between bud and air
δ_{bl}	Boundary layer thickness

Chapter 1

General Introduction

In higher plants, establishment, growth and reproduction are primarily dependent on the continuous activity of two different groups of undifferentiated cells, the root and shoot meristems. Shoot and root meristems are driving the above- and below-ground organ generation respectively (Barlow 1989).

In indeterminate plant species the shoot apical meristem (SAM) is continuously producing shoot modules, i.e. the phytomeres (Barthélémy and Caraglio 2007). In *Cucumis sativus* L. (cucumber) plants, for example, a phytomer during the vegetative stage mainly consists of a leaf, an internode, an axillary meristem and a tendril while during the generative phase, phytomeres additionally consist of flower meristems. The SAM is, hence, the fountain and simultaneously the architect of the shoot.

The SAM is a dome of cells (Fig. 1) usually surrounded by the already successively formed and folded primordial leaves. This creates a distinct structure that resides on the top of the shoot, the apical bud (Fig. 2). The formation of a new phytomer on the shoot is presignified when a new leaf primordium is initiated (projected) on this dome (Fig. 1). The fundamental importance of leaf initiation for plant growth and development led to the in-depth, from cell-to-molecule, exploration of this process and the unravelling of its complex component-mechanisms (e.g. Lyndon 1994; Fleming *et al.* 1997; Ha *et al.* 2010; Besnard *et al.* 2011).

Leaf initiation is taking place through the continuous proliferation of pluripotent cells in the SAM and the synchronous transition in the fate of a group of these pluripotent cells to determinate cells (Byrne 2012). The change in fate is associated with changes in gene expression and new patterns of cell division and expansion (Golz 2006). As these cells proliferate, new axes of growth are established lateral to the SAM resulting in an outgrowth (leaf primordium) from

the flanks of the SAM (Golz 2006). The direction of this outgrowth and thus the positioning of the new leaf primordium on the SAM in relation to the earlier initiated primordia (i.e. phyllotaxis) are basically determined by auxin gradients (Reinhardt *et al.* 2003). Briefly, auxin once in the SAM, is absorbed by the existing developing primordia which are acting as auxin sinks depleting auxin from the surrounded tissue (Reinhardt *et al.* 2003). Therefore, auxin accumulates in the region of the SAM furthest from the previously formed primordia and, as a consequence, when auxin passes a critical threshold in this region a new primordium is initiated (Golz 2006). Consequently, leaf initiation and its spatial arrangement are determined by a complex signaling network between the SAM and the earlier initiated leaf primordia (Ha *et al.* 2010). While the spatial pattern of leaf initiation is mainly a matter of intrinsic plant decisions and less a matter of extrinsic (environmental) cues (Kuhlemeier 2007), the rate in which the process of leaf initiation is repeated is highly dependent on the environment (e.g. Hussey 1963a; Granier *et al.* 2002).

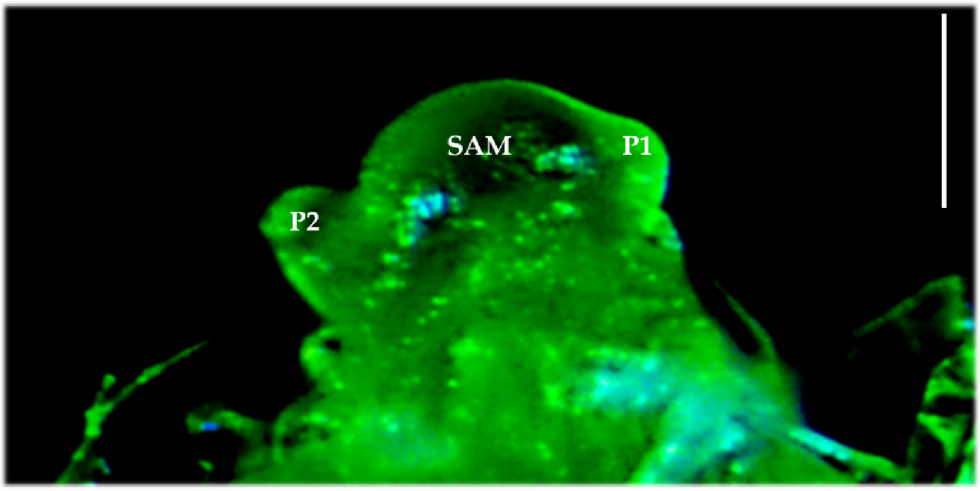

Fig. 1. Stereo-microscopic image of the shoot apical meristem (SAM), the latest (P1) and the earlier (P2) initiated leaf primordia after the dissection of the earlier initiated primordial leaves and tendrils in a cucumber plant. The scale bar represents 0.1mm.

Leaf initiation rate (LIR; number of leaves initiated per day) is a widely-used measure of the number of leaves as well as the number of phytomeres

initiated over time. Hence, LIR is a critical feature for plant architecture, plant leaf area, and therefore plant growth (Ackerly *et al.* 1992; Sussex and Kerk 2001). Over the last century, the common assumption was that LIR is mainly driven by air temperature (T_{air}), which stands until today. This study primarily focuses on linking LIR to the aerial environment and states that T_{air} is not the whole story in this linkage.

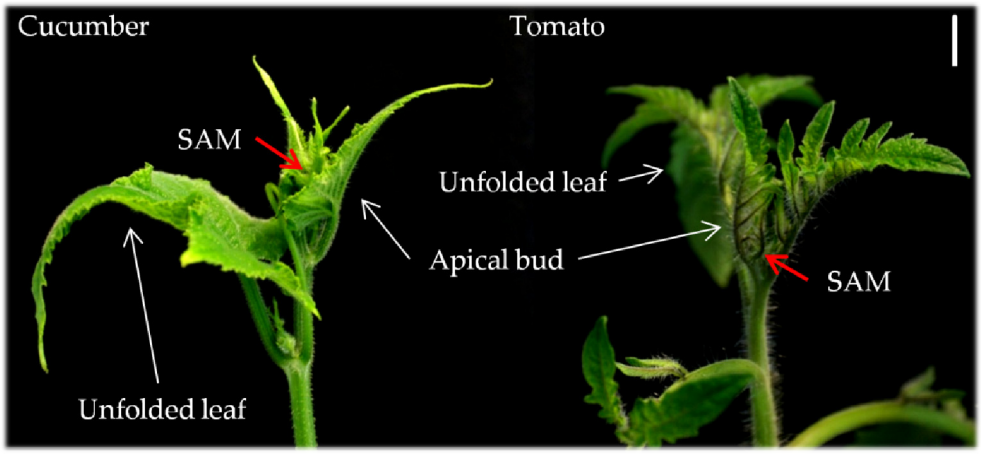

Fig. 2. Image of the apical bud in a young generative *Cucumis sativus* L. (cucumber) plant (left) and in a young generative *Solanum lycopersicum* L. (tomato) plant (right). The scale bar represents 1cm.

1.1. Leaf initiation rate and temperature

Unlike often implicitly assumed not air temperature but plant temperature, the temperature actually perceived by the plants is the key modulator of plant development and therefore of crop yield (Atkinson and Porter 1996; Craufurd and Wheeler 2009). Shoot apical meristem temperature ($T_{meristem}$) is the key-modulator of LIR (Jamieson *et al.* 1995; Granier and Tardieu 1998; Granier *et al.* 2002). LIR linearly increases with the averaged diel $T_{meristem}$ in a species-specific range (Parent and Tardieu 2012) defined by a low (base) and a higher (optimum) threshold temperature (Atkinson and Porter 1996). In fast-developing crop species LIR shows steep responses to temperature within this range (*Cucumis sativus* L., Marcelis 1993b; *Pisum sativum* L., Turc and Lecoeur 1997; *Helianthus annuus* L., Granier and Tardieu 1998; *Cucumis melo* L., Baker and Reddy 2001). Below the base temperature

leaf initiation ceases (Porter and Semenov 2005). Above the optimum temperature, LIR decreases (Craufurd *et al.* 1998) until leaf initiation ceases again above a maximum temperature (Porter and Semenov 2005). Despite its strong effect on LIR, $T_{meristem}$ is hardly ever quantified. Instead, T_{air} is often used as an easy-to-quantify approximation of $T_{meristem}$. However, the use of T_{air} in studying and predicting the effects of $T_{meristem}$ on LIR may be inaccurate (Jamieson *et al.* 1995; Vinocur and Ritchie 2001) because $T_{meristem}$ may largely deviate from T_{air}.

1.1.1. Shoot apical meristem temperature: is it always equal to air temperature and similar across species?

Most plant species do not sufficiently control their temperature to maintain thermal homeostasis. Plant temperature fluctuates depending on the environment (Jones 1992). Therefore, plants are 'classified' as poikilotherms (i.e. organisms whose body temperature fluctuates in response to their environment; McNaughton 1972; Körner 2006). Misinterpretation of this term probably triggered the to-date common assumption that plant temperature is always and solely following air temperature ignoring the numerous studies indicating that this is not actually the case (e.g. Geller and Smith 1982; Wilson *et al.* 1987).

The temperature of a plant organ is the net outcome of the heat exchange between the organ and its environment. Besides T_{air}, other environmental variables like radiation, wind speed, and vapour pressure deficit are strongly involved in the heat exchange processes between plant organs and their environment (Nobel 2009). Therefore, fluctuations in these environmental factors may also contribute to deviations of $T_{meristem}$ from T_{air} in nature, field crop cultivation and protected crop cultivation (Wilson *et al.* 1987; Faust and Heins 1998; Guilioni *et al.* 2000). Approximation of $T_{meristem}$ with T_{air} under these environments could result in an over- or underestimation of the effect of $T_{meristem}$ on LIR, as well as incorrect acknowledgment of the impact other environmental factors per se (e.g. light intensity, day length) as influential for LIR.

Plants despite being poikilotherms and therefore having low thermal homeostatic ability can partly adjust their temperature (thermoregulation). Thermoregulation is one of the main drivers of the evolution of plant organ structure and its function (e.g. transpiration; Nicotra *et al.* 2011; Pincebourde and Woods 2012). Plants evolutionary adjusted their structure and function to avoid

harmful organ temperatures (Smith 1974; Meinzer and Goldstein 1985; Nobel *et al.* 1986; Leigh *et al.* 2012). Organ structure and function are therefore important players in organ thermoregulation (Raschke 1960). Taking into consideration the large interspecific variation of organ structure and function, it can be speculated that different species perceive different organ temperatures in the same environment. Indeed, studies on leaf temperature revealed that different species perceive different leaf temperatures when subjected to the same environmental conditions (Geller and Smith 1982; Hatfield and Burke 1991) due to interspecific variation in leaf traits like orientation, absorptance of shortwave radiation (Geller and Smith 1982), and transpiration (Hatfield and Burke 1991). However, knowledge is lacking for more complex plant structures such as apical buds.

Shoot apical meristems are enclosed within apical buds. The apical bud is a complex structure usually composed of folded primordial organs that were lately formed by the meristem (Fig. 2). The enclosure of the SAM within the bud suggests that meristem microenvironment and therefore $T_{meristem}$ are strongly related to the bud structure and function. The type, number, size, shape, and arrangement of the organs comprising the bud vary enormously between species (Bell and Bryan 2008), for example, between cucumber and tomato plants (Fig. 2). Functional traits like transpiration capacities of such complex structures are usually difficult to quantify and their contribution to heat exchange remains uncertain. Therefore, species differing in bud structure and function may experience different $T_{meristem}$ under the same environments.

The response of $T_{meristem}$ to environmental variables has never been quantified in a systematic way under moderate environments and little is known on differences in $T_{meristem}$ between crop species grown in the same environment. Accordingly, the link between $T_{meristem}$ and the structural-functional aspects of the bud is still rather unspecified.

1.1.2. Shoot apical meristem: the only site of temperature perception regarding leaf initiation?

$T_{meristem}$ may deviate from T_{air} and across species. Additionally, within a plant, temperature is not always uniform either. Vertical intra-plant temperature differences, mainly caused by vertical microclimatic differences, were observed in nature (Gibbs and Patten 1970), field crop cultivation (Gardner *et al.* 1981) and in

protected cultivation (Kempkes and van de Braak 2000; Li *et al.* 2014). In contrast to other plant microclimate heterogeneities (e.g. light gradients; Pons *et al.* 2001), the effects of such temperature heterogeneities on plant development have hardly been studied.

The top of the shoot may be subjected to varying solar radiation (Gibbs and Patten 1970), wind speeds (Tuzet *et al.* 1997) and/or thermal radiation (Leuning and Cremer 1988) than the lower part of the shoot due to the higher exposure of the top shoot to the extra-canopy environment. Therefore, $T_{meristem}$ and the temperature of the surrounding folded leaves forming the apical bud may considerably deviate from the temperature of the rest of the plant (T_{plant}).

Previous studies suggested that it is more accurate to link LIR to $T_{meristem}$ instead of T_{air} (Jamieson *et al.* 1995; Granier and Tardieu 1998). To the best of our knowledge, there is no experimental evidence proving that LIR is not also influenced by plant temperatures other than $T_{meristem}$. In several cases, environmental cues (e.g. temperature, light intensity, ambient CO_2 concentration) are sensed by the mature plant tissues (e.g. leaves) and systemic signals from these tissues are mediating developmental changes in young tissues (Lake *et al.* 2001; Coupe *et al.* 2006; Gorsuch *et al.* 2010). These systemic signals, such as sugars and hormones (Coupe *et al.* 2006), are potentially acting as a warning system to enable young tissues to cope with their current environment (Gorsuch *et al.* 2010). It is also worth mentioning that LIR may be highly influenced by increased number of sinks (Marcelis 1993b) or leaf (source) removal (Hussey 1963b) suggesting a systemic control of LIR via altered resource (carbon) availability. This strengthens the notion hypothesis that LIR may not only be related to the local perception of temperature in the SAM or the apical bud, in this case by $T_{meristem}$, but also be influenced by temperatures of other plant parts. If so, plants subjected to temperature differences between the apical bud and the rest of the plant may show 1) LIR that is not corresponding solely to $T_{meristem}$, and integrating this possible local response to plant level, 2) phenotypes that are beyond expectation. The response of LIR to such intra-plant temperature heterogeneities and the possible effects of this response on plant phenotype did not yet attract attention. Accordingly, SAM cannot be securely nominated as the only site of temperature perception regarding leaf initiation.

1.2. Leaf initiation rate and light

Photosynthetic photon flux density (PPFD) was also reported as influential for LIR (Hussey 1963a; Newton 1963) as well as for other developmental processes (e.g. root meristematic development; Freixes *et al.* 2002). However, PPFD effects on LIR are still ambiguous. Numerous studies reported either positive (Hussey 1963a; Newton 1963; Pieters 1985; Marcelis 1993b; Cookson *et al.* 2005) or no relation of PPFD and LIR (Beinhart 1963; Heuvelink and Marcelis 1996).

Species mobilize different strategies, and therefore, different physiological and morphological traits to adapt to their ever changing light environment (Valladares and Niinemets 2008). Therefore, the differences observed between studies may be the result of differences in the sensitivity of leaf initiation of different species to PPFD.

Besides these ecophysiological reasons, methodological differences may well be a reason for the deviations observed in earlier studies of LIR responses to PPFD. Firstly, mostly air temperature (T_{air}) and to a lesser extent leaf temperature (T_{leaf}) were used as approximations of $T_{meristem}$. $T_{meristem}$ may deviate from T_{air} depending on other environmental factors, that are also influencing meristem heat budget, like radiation (Wilson *et al.* 1987). Secondly, it is usually assumed that the light quality (i.e. spectral distribution of photon flux density) is homogeneous when manipulating PPFD. Hence, it is often not quantified. However, PPFD manipulation may cause substantial changes in the light quality perceived by the plants depending on the methodology followed (e.g. the use of nettings that do not intercept all the wavelengths to an equal extent; Poorter *et al.* 2012). Light quality is highly influencing leaf development and functionality (Hogewoning *et al.* 2010; Savvides *et al.* 2012). Specifically, variation in red: far red ratio (Carabelli *et al.* 2007) and blue light fluence-rate under constant PPFD (Christophe *et al.* 2006) were reported as influential for leaf appearance and subsequent leaf expansion. Consequently, controversies between studies on the responses of LIR to PPFD may also be due to variation in light quality during experimentation. Thirdly, the rates at which successive leaves appear (LAR; become visible to the naked eye) or unfold (LUR) are usually used as approximates of LIR to avoid laborious and destructive micro-stereoscopic observations to accurately determine LIR. It was already shown that the early stages of leaf expansion (i.e. leaf initiation and leaf early growth) are correlated processes (Cookson *et al.* 2005). However, this

correlation does not necessarily imply equality between LIR, LAR and LUR. Previous studies suggested equality between LIR, LAR and LUR on the long-term (Heuvelink and Marcelis 1996) but inequality on the short-term (e.g. early vegetative stage; Newton 1963). Consequently, it is still debatable whether LAR and/or LUR can be used as precise approximates of LIR under different PPFDs.

The response of LIR, LAR or LUR to PPFD may be related with the carbohydrate availability in the local tissue. Carbohydrates, despite being the substrate for growth, are also mediating the responses of several developmental and growth processes to light (Freixes *et al.* 2002; Moore *et al.* 2003). The SAM and the surrounding-folded developing leaves (i.e. apical bud) are considered as sinks (i.e. imported carbohydrates are the main resource for growth and maintenance; Ho 1988). Sink-to-source transition in leaves begins shortly after unfolding (Turgeon 1989). The early stages of leaf expansion are strongly dependent on local carbohydrate availability and metabolism (Pantin *et al.* 2012). Therefore, it can be hypothesized that the PPFD responses of developmental and growth processes taking place within the apical bud are related with the local carbohydrate availability and utilization (metabolism). However, the relation between light and carbohydrate availability in the apical bud even though suggested (Hussey 1963b; Newton 1963; Marcelis 1993b) has not been yet investigated.

The rate at which leaves/phytomeres are initiated can be an adaptive trait of plants to changes in PPFD. The controversy between studies on the relation between LIR and light strengthens the necessity to further unravel the relation between LIR and PPFD.

Key objectives of this thesis

It can be argued, that relating leaf initiation rate solely to air temperature may lead to substantial misapprehension of the effects of the different components of the aerial environment on LIR. These components may 1) influence T_{meristem} (e.g. solar radiation) and therefore LIR and 2) affect LIR without influencing T_{meristem} (e.g. PPFD and microclimatic gradients inducing intra-plant temperature heterogeneities). Hence, the central aim of this thesis is to more accurately link leaf initiation rate to the aerial environment. This central aim can be split in several key objectives:

- Unravelling the contribution of the different aerial environmental variables as well as the contribution of apical bud heat-exchange-related traits on $T_{meristem}$.
- Revealing whether the apical bud is the sole site of temperature perception regarding LIR even under intra-plant temperature differences between the apical bud and the rest of the plant.
- Determining the effects of the intra-plant temperature differences between the apical bud and the rest of the plant on plant phenotype.
- Unravelling the relation between LIR and PPFD as well as the possible relation between the potential effects of PPFD on LIR and carbon availability.

Contents of this thesis

Chapter 2 describes how meristem temperature deviates from air temperature in fast-growing crop species under moderate environments by systematically changing environmental variables such as radiation, wind speed, vapour pressure deficit and air temperature and unravels the contribution of bud structure and function to $T_{meristem}$ in cucumber and tomato plants.

Chapter 3 describes the response of LIR to bud-plant temperature differences created using a custom-made device that is altering T_{bud} in cucumber plants.

Chapter 4 shows the critical alterations in plant phenotype, from leaf- to plant-level due to bud-plant temperature differences in cucumber plants.

Chapter 5 shows the response of LIR, LAR and LUR to (changes in) light intensity in cucumber and tomato plants in relation to the local (bud) carbohydrate availability.

Chapter 6 is the general discussion. The findings described in chapters 2 to 5 are brought together to give 1) a holistic answer to the question 'why air temperature is not the whole story when linking leaf initiation to the aerial environment', 2) to discuss the implications in the study of plant ecophysiology and plant growth modelling but also the practical implications for plant productions systems, 3) to discuss future perspectives in the study of leaf initiation in response to the environment and 4) to initiate the critical matter of plant temperature heterogeneities and their impacts on plant phenotype.

Chapter 2

Meristem temperature substantially deviates from air temperature even in moderate environments: Is the magnitude of this deviation species-specific?

Abstract

Meristem temperature ($T_{meristem}$) drives plant development but is hardly ever quantified. Instead, air temperature (T_{air}) is usually used as its approximation. Meristems are enclosed within apical buds. Bud structure and function may differ across species. Therefore, $T_{meristem}$ may deviate from T_{air} in a species-specific way. Environmental variables (air temperature, vapour pressure deficit, radiation, and wind speed) were systematically varied to quantify the response of $T_{meristem}$. This response was related to observations of bud structure and transpiration. Tomato and cucumber plants were used as model plants since they are morphologically distinct and usually growing in similar environments. $T_{meristem}$ substantially deviated from T_{air} in a species-specific manner under moderate environments. This deviation ranged between -2.6 and 3.8 ^{o}C in tomato and between -4.1 and 3.0 ^{o}C in cucumber. The lower $T_{meristem}$ observed in cucumber was linked with the higher transpiration of the bud foliage sheltering the meristem when compared with tomato plants. We here indicate that for properly linking growth and development of plants to temperature in future applications, for instance in climate change scenarios studies, $T_{meristem}$ should be used instead of T_{air}, as a species-specific trait highly reliant on various environmental factors.

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Introduction

Plant temperature is a key modulator of plant development and therefore of crop yield (Atkinson and Porter 1996; Craufurd and Wheeler 2009). Leaf initiation rate (LIR) is a measure of the number of leaves as well as the number of phytomeres (leaf, internode, and axillary bud) formed by the shoot apical meristem in time. Consequently, LIR is a strong determinant of plant architecture, plant leaf area, and therefore plant growth in time (Ackerly *et al.* 1992; Sussex and Kerk 2001). $T_{meristem}$ is the key-modulator of LIR (Jamieson *et al.* 1995; Granier and Tardieu 1998; Granier *et al.* 2002). LIR is positively and linearly related with the averaged diel $T_{meristem}$ in a species-specific range (Parent and Tardieu 2012) defined by a low (base) and a higher (optimum) threshold temperature (Atkinson and Porter 1996). In fast-developing crop species LIR shows steep responses to temperature within this range (Marcelis 1993b; Turc and Lecoeur 1997; Granier and Tardieu 1998; Baker and Reddy 2001). Below the base temperature leaf initiation ceases (Porter and Semenov 2005). Above the optimum temperature, LIR decreases (Craufurd *et al.* 1998) until leaf initiation ceases again above a maximum temperature (Porter and Semenov 2005). Despite its strong effect on LIR, $T_{meristem}$ is hardly ever quantified. Instead, T_{air} is used as an easy-to-quantify approximation of $T_{meristem}$. However, the use of T_{air} in studying and predicting the effects of $T_{meristem}$ on LIR may be inaccurate (Jamieson *et al.* 1995; Vinocur and Ritchie 2001).

$T_{meristem}$ may vary largely from T_{air}. Most of the plant species are considered as poikilotherms; their temperature fluctuates in response to their (thermal) environment. The temperature of a plant organ is the net outcome of the heat exchange between the organ and its environment. Besides T_{air}, other environmental variables like radiation, wind speed (U), and vapour pressure deficit (VPD) are strongly involved in the heat exchange processes (Nobel 2009). R_{net} (the net radiation absorbed by a body) is a strong determinant of $T_{meristem}$ especially at low U where convective heat exchange between the air and plant surfaces is rather low (Wilson *et al.* 1987; Guilioni *et al.* 2000). For example, in a sheltered (low height) montane vegetation at high R_{net}, $T_{meristem}$ was 15 ^{o}C higher than T_{air} (Wilson *et al.* 1987). In a giant rosette species, $T_{meristem}$ was more than 5 ^{o}C lower than T_{air} during an Andean spring clear night (negative R_{net}; Smith 1974). $T_{meristem}$ deviated from T_{air}

also in crop species. At high R_{net}, $T_{meristem}$ in *Zea mays* was 7 °C higher than T_{air} (Guilioni *et al.* 2000). In *Cantharanthus roseus* growing in a glasshouse $T_{meristem}$ was 5 °C lower than T_{air} when the glazing material temperature was 16 °C below T_{air} at night (Faust and Heins 1998). In addition, increased VPD at low U resulted in decreasing $T_{meristem}$ at night in *Catharanthus roseus* (Faust and Heins 1998). Environments, especially with low U may then induce substantial deviations of $T_{meristem}$ from T_{air} ([$T_{meristem}$ - T_{air}]) depending mainly on R_{net} and VPD. Approximation of $T_{meristem}$ with T_{air} under these environments could result in an over- or underestimation of the effect of $T_{meristem}$ on LIR which could lead to incorrect acknowledgment of other factors per se (e.g. light intensity, daylength) as influential for LIR. The occurrence of substantial [$T_{meristem}$ - T_{air}] justifies the development of species-specific heat exchange models on predicting $T_{meristem}$ (see e.g. Cellier *et al.* 1993; Faust and Heins 1998; Guilioni *et al.* 2000; Shimizu *et al.* 2004).

Plants despite being poikilotherms and therefore having low thermal homeostatic ability can partly adjust their temperature (thermoregulation). Thermoregulation is one of the main drivers of the evolution of plant organ structure and function (e.g. transpiration; Nicotra *et al.* 2011; Pincebourde and Woods 2012). Plants evolutionary adjusted their structure and function to avoid harmful organ temperatures (Smith 1974; Meinzer and Goldstein 1985; Nobel *et al.* 1986; Leigh *et al.* 2012). Organ structure and function are therefore important players in organ thermoregulation (Raschke 1960). Taking into consideration the large interspecific variation of organ structure and function it can be speculated that different species perceive different organ temperatures in the same environment. Indeed, studies on leaf temperature revealed that different species perceive different leaf temperatures when subjected to the same environmental conditions (Geller and Smith 1982; Hatfield and Burke 1991). Interspecific variation in leaf traits like orientation, absorptance of shortwave radiation (Geller and Smith 1982), and transpiration (Hatfield and Burke 1991) was strongly related to the diverse leaf temperatures observed among the species studied. However, knowledge is lacking for more complex plant structures such as apical buds.

Shoot apical meristems are groups of cells (domes) enclosed within apical buds. The (apical) bud is a complex structure usually comprising of folded primordial organs that were lately formed by the meristem. The enclosure of the

meristem within the bud suggests that meristem microenvironment and therefore $T_{meristem}$ are strongly related to the bud structure and function. The type, number, size, shape, and arrangement of the organs comprising the bud vary enormously between species (Bell and Bryan 2008). However, functional traits like transpiration capacities of such complex structures are usually difficult to quantify and their contribution to heat exchange remains uncertain. Consequently, species differing in bud structure and function may experience different $T_{meristem}$ under the same environments.

Grace (2006) indicated that for a proper estimation of the effect of climate change on the rate of plant growth, it is not sufficient to assume that physiology is driven by T_{air}. Indeed, connecting organismal physiology to air rather than to body temperature may lead to erroneous interpretations of the potential effects of climate change, as suggested by ecological studies on leaf-air temperature deviations in plants at global scale (Linacre 1967; Helliker and Richter 2008). Furthermore, different ectothermic animal species (i.e. their body temperature hardly depends on internal heat sources) sharing the same microhabitats show different body temperatures (Broitman *et al.* 2009). According to Broitman *et al.* (2009), this suggests that habitat temperatures alone do not determine the present and future distribution as well as the abundance of these species, but body temperatures may well enhance the understanding and prediction of these ecological traits. The same reasoning seems applicable for different plant species growing in identical environments indicating the ecological importance of investigating possible interspecific differences in organ temperatures.

The response of $T_{meristem}$ to environmental variables has never been quantified in a systematic way and little is known on differences in $T_{meristem}$ between crop species grown in the same environment. Accordingly, the link between $T_{meristem}$ and the structural-functional aspects of the bud is still rather unspecified. In this study we aim to 1) quantify how $T_{meristem}$ deviates from T_{air} in fast-developing crop species under moderate environments and 2) unravel the contribution of bud structure and function to $T_{meristem}$. Tomato and cucumber plants were used as model systems. They are two morphologically distinct crop species usually grown and studied under similar protected environments. Effects of the environmental variables on $T_{meristem}$ were analysed in a systematic way. The

response to the environment was related to heat exchange-related, structural-functional traits of the apical bud.

Materials and methods

Plant material and growth conditions

Cucumber (*Cucumis sativus* L. cv. Venice RZ) and tomato (*Solanum lycopersicum* L. cv. Cappricia RZ) plants were grown in a climate room (length: 5 m; width: 3 m; height: 2.5 m) at 20 ^{o}C T_{air}, 70% relative humidity (RH; VPD = 0.7 kPa), 0.2 m s^{-1} U and ambient [CO_2]. The plants were illuminated by 16 SON-T lamps (MASTER GreenPower CGT 400W E40 1SL; Royal Philips Electronics N.V., Amsterdam, The Netherlands) at a photosynthetic photon flux density (PPFD) of 450 µmol m^{-2} s^{-1} during 16 h photoperiod. Plants were watered with nutrient solution (EC = 2 dS m^{-1}, pH = 5.0 - 5.5) in an ebb and flood irrigation system. Tomato seeds were sown a week earlier than cucumber to achieve the same developmental stage at the start of the treatments as cucumber plants are developing faster than tomato plants. Four weeks after cucumber plants emerged, when the 7th leaf had unfolded (away from the bud) in both the species, plants were simultaneously subjected to a range of environmental conditions.

Systematic variation of environmental variables

R_{net}, T_{air}, and VPD were independently varied in short-term (diel steps). One of these three environmental variables was varied at a time, while the other two variables were fixed (Table 1); the set-point values for R_{net}, T_{air}, and VPD were 180 W m^{-2}, 20 ^{o}C, and 0.7 kPa respectively (Table 1). All experiments were performed at two U's (0.2 and 0.6 m s^{-1}). Three batches of both the plant species were used to investigate the effects of each of the three environmental variables on $T_{meristem}$.

T_{air} was varied from 16 to 32 ^{o}C in five diel (constant temperature per 24h) steps. VPD was varied from 0.3 to 1.2 kPa (by varying RH) in five diel steps. R_{net} (the net radiation absorbed by a black body) was varied from -80 to 320 W m^{-2} in four steps. The second step was the night period (R_{net} = 0 W m^{-2}, PPFD = 0 µmol m^{-2} s^{-1}), while the third step was the day period of the control treatment. The highest

radiation step was achieved by doubling the number of SON-T lamps from 15 at control conditions (third level; R_{net} = 180 W m^{-2}, PPFD = 445 μmol m^{-2} s^{-1}) to 30 (fourth level; R_{net} = 320 W m^{-2}, PPFD = 850 μmol m^{-2} s^{-1}). The SON-T lamps were isolated from the climate cell by a glass ceiling which enabled the separate convective cooling of the lamps. The glass ceiling temperature ($T_{ceiling}$; ~32 °C) was higher than T_{air} at control conditions (during day) and increased further (to ~37 °C) with increasing light intensity (double number of lamps) and at night was equal to T_{air}. In order to create the lowest R_{net} step (R_{net} = -80 W m^{-2}, PPFD = 0 μmol m^{-2} s^{-1}), well below the control night conditions (or in other words, simulate the conditions induced by a clear night sky) a metallic basin (0.75x1.5 m) filled with ice was placed 0.2 m above the plants while T_{air} and VPD around the plants were measured and found not influenced by the cold (~5 °C) surface of the basin. The low U was the control, while the high U was achieved by a network of computer fans connected in parallel and controlled by a power supply with adjustable voltage.

Table 1. Overview of the environmental variables in the three experiments. In each experiment air temperature (T_{air}), vapour pressure deficit (VPD), or net radiation (R_{net}) was varied while other environmental variables were fixed. All treatments were performed at two levels of wind speed (U).

Experiments	T_{air} (°C)	VPD (kPa)	R_{net} (W m^{-2})	U (m s^{-1})
T_{air}	16, 20, 24, 28, 32	0.7	180 (day) / 0 (night)	0.2 & 0.6
VPD	20	0.3, 0.5, 0.7, 0.9, 1.2	180 (day) / 0 (night)	0.2 & 0.6
R_{net}	20	0.7	-80, 0, 180, 320	0.2 & 0.6

Climatic measurements

T_{air} and RH were monitored by a temperature/humidity sensor (1400-104; LI-COR Inc., Lincoln, NE, USA) placed in an aspirated climate monitoring box in the centre of the climate room and data were logged by a data-logger (LI-1400; LI-COR Inc., Lincoln, NE, USA). The temperature/humidity sensor was compared with the thermocouples used for the plant temperature measurements (see below) in darkness (to avoid radiation effects on temperature sensing) under varied T_{air}. No significant differences were found in temperature sensing.

Shortwave radiation (R_{SW}; in the range of 380-2800 nm) and R_{net} (in the range of 0.2-100 μm) absorbed by a black body were measured at plant height by a pyranometer (GSM 10.7; Adolf Thies GmbH & Co. KG, Gottingen, Germany) and a net radiometer (NR LITE; Kipp & Zonen, Delft, The Netherlands) respectively. Radiation data were recorded by a data-logger (ADC-24; Pico Technology, Cambridgeshire, UK). Data acquisition software (Picolog; Pico Technology, Cambridgeshire, UK) was used to monitor and record the climate and meristem temperatures. R_{net} is the sum of R_{SW} (<2800 nm) and longwave radiation (>2800 nm) absorbed by the black body minus the longwave radiation emitted. The quantification of R_{net} and R_{sw} enables the estimation of the net (absorbed minus emitted) longwave radiation absorbed by a black body (R_{LW}). The lamps used do not emit radiation below 380 nm therefore the difference in the lower part of the measuring range between the pyranometer and the net radiometer can be ignored. U was quantified by a 3D ultrasonic anemometer (WindMaster™; Gill Instruments LTD, Hampshire, UK) at the height of the bud.

Meristem temperature measurements

K-type fine-wire thermocouples with a spherical junction (diameter close to 0.5 mm) were constructed and calibrated by insertion in 0 °C (ice bath) and 100 °C (boiling point) water bath under constant atmospheric pressure (101.3 kPa). The thermocouples were supported by a thicker flexible cable coupled to lab stands in order to assure their position when attached to the plant tissue and they were connected to data-loggers (USB TC-08; Pico Technology, Cambridgeshire, UK) for temperature monitoring and recording. $T_{meristem}$ was monitored by gently inserting the thermocouple in the bud as close as possible to the centre where the meristem is located. The position of the thermocouples was regularly checked to assure the validity of the measurements. $T_{meristem}$ as well as the climate conditions were recorded every 30 seconds throughout the treatments and only the steady-state temperatures (the average of 1 hour recordings after reaching steady-state) were used in the analyses. Measurements were performed on 12 plants per species.

A thermal imaging camera (FLIR B660; FLIR Systems Inc., Wilsonville, OR, USA) was used for supplementary measurements and visualization of tissue temperatures at selected environments. Plant tissue thermal emissivity was set at

0.95 (Jones 2004) and the climate conditions (T_{air} and RH) at the time of imaging were incorporated for thermal image analysis.

Apical bud transpiration

A portable gas exchange system (Fig. 1*a*; LI-6400; LI-COR Inc., Lincoln, NE, USA) connected to a custom-made chamber was used to measure the diurnal and nocturnal transpiration rates of the bud (E_{bud}) in a range of VPD. The chamber consisted of a transparent, hollow PVC sphere comprised by two hemispheres (Fig. 1*c*). The sphere was connected to the LI-6400 on the sample tubing between the main body and the infrared gas analysers located in the measuring head (below the leaf chamber) of the system (Fig. 1*b*). Adjustments were made to isolate the infrared gas analyser sample cell from the LI-6400 leaf chamber in order to use the LI-6400 as a stand-alone gas analyser. The lower part of the leaf chamber was replaced by a manifold (Fig. 1*d*; Sample cell outlet manifold; LI-COR Inc., Lincoln, NE, USA) and adhesive tape was used to cover the holes of the lower leaf chamber manifold to prevent air circulation in the upper part of the leaf chamber (Fig. 1*d*).

The openings of the two hemispheres were covered with parafilm for better insulation. The sphere was checked for CO_2 and H_2O absorption and leakages. The sphere was also tested for transmitted light intensity and quality. The sphere allowed ~90% light transmittance without affecting the light spectrum.

Intact buds were inserted through a small circular opening in the bottom of the sphere (Fig. 1*c*). The opening and the stem part at that point were covered by 'sticky tac' (Pritt; Henkel AG & Co. KGaA, Dusseldorf, Germany) to avoid leakages but also stem damage.

The T_{air} inside the sphere and the $T_{meristem}$ were continuously recorded during the measurements by thermocouples. $T_{meristem}$ was used as an approximation of the bud temperature assuming the absence of temperature gradients within the bud. Bud to air vapour pressure difference ($VPD_{bud\text{-}air}$; kPa; Eqn. 1) was calculated from the measurements of T_{air}, $T_{meristem}$, and RH by assuming 100% RH inside the bud. The equations for the estimation of $VPD_{bud\text{-}air}$ were adopted from Jones (1992):

$$VPD_{bud-air} = e_{bud} - e_{air} \qquad \textbf{(Eqn. 1)}$$

$$e_{bud} = 0.613 \exp\left(\frac{17.502\, T_{meristem}}{240.97+T_{meristem}}\right) \text{ and}$$

$$e_{air} = \frac{RH*e_s}{100} \text{ , where } e_s = 0.613 \exp\left(\frac{17.502\, T_{air}}{240.97+T_{air}}\right)$$

Where, $VPD_{\text{bud-air}}$ is the difference between the saturation vapour pressure at bud surface (e_{bud}; kPa) and the vapour pressure of the surrounding air (e_{air}; kPa). e_{bud} was estimated based on T_{meristem} (°C) while e_{air} was estimated based on the measured T_{air} (determinant of the air vapour pressure at saturation; e_s) and RH. Measurements were performed on four plants per species when the 7th leaf had unfolded. The E_{bud} measured was then divided (normalized) by the leaf area (both the adaxial and abaxial leaf surface) of the leaves contained in the bud ($E_{\text{bud area}}$).

Apical bud structure

The number of leaves contained in the bud was quantified by observations (60-310X magnification) using a microstereoscope (Wild M7S, Wild Heerbrugg Ltd., Heerbrugg, Switzerland). The measurements took place on 8 plants per species when the 7th leaf had unfolded. The comprising leaves were dissected and imaged and the total contained leaf area (the sum of adaxial and abaxial surface area of each leaf) was quantified using ImageJ (Schneider *et al.* 2012) for the normalization of the transpiration rates. Images on the external and internal apical bud structure were taken by respectively using a single lens reflex digital camera (EOS 1000D; Canon Inc., Tokyo, Japan) and the microstereoscope connected to a digital imaging camera (Nikon DXM-1200; Nikon Co., Tokyo, Japan).

Statistical analysis

A linear model (Genstat 15th ed.; VSN International Ltd, Hemel Hempstead, UK) was fitted to the data using T_{meristem} or [T_{meristem}-T_{air}] as response variate. The environmental variables (T_{air}, VPD, R_{net} and U) and species were selected as explanatory variates (when $P < 0.05$). All the possible interactions ($P < 0.05$) between the explanatory variates were tested.

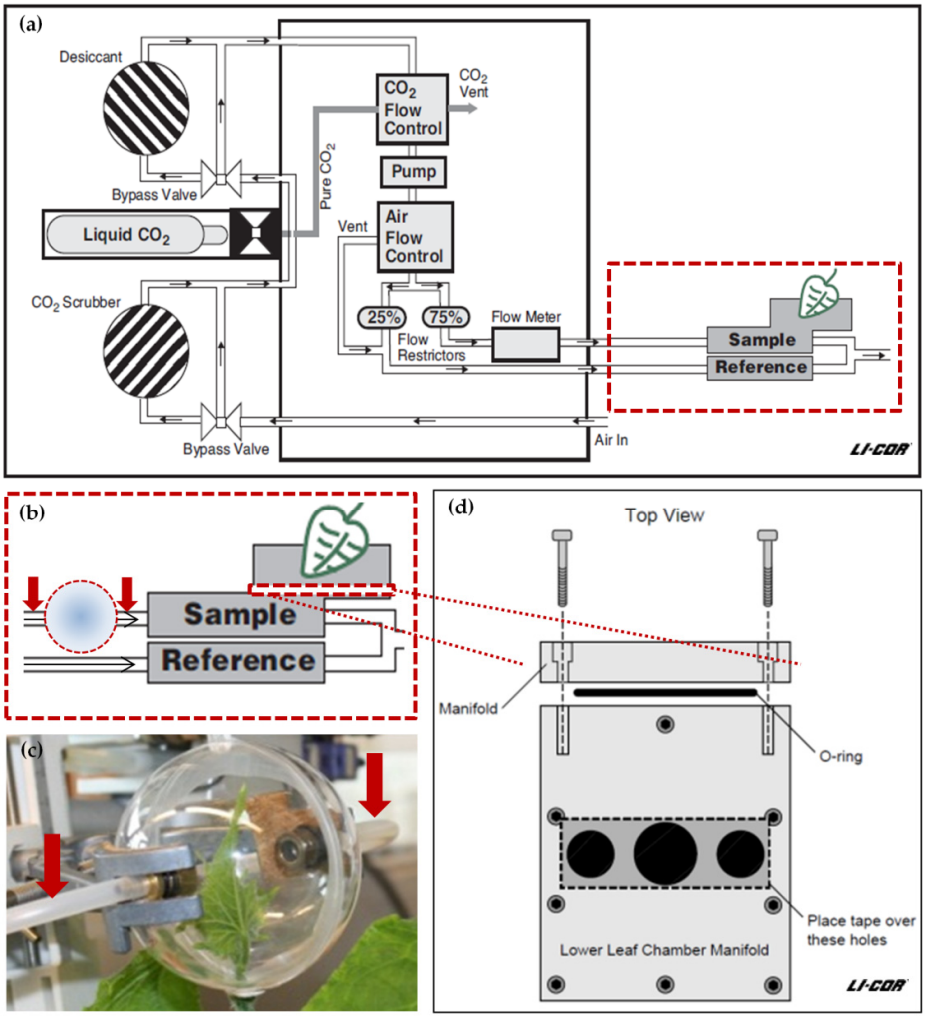

Fig. 1. Set up of measurements of apical bud transpiration. The LI-6400 portable gas exchange system (*a*; schematic representation adopted from LI-6400 manual, LI-COR Inc.) in combination with a custom-made spherical chamber (*c*) was used for apical bud transpiration measurements. The spherical chamber was connected on the sample tubing system (arrows; *b* and *c*). The leaf chamber, located on the measuring head of the LI-6400, was excluded from the air flow system in order to use the LI-6400 as a stand-alone gas analyser by 1) replacing the lower part of the chamber with a sample cell outlet manifold (*d*; schematic representation adopted from sample manifold installation instructions, LI-COR Inc.) and 2) covering the holes of the lower leaf chamber manifold with adhesive tape to prevent air circulation in the upper part of the leaf chamber (*d*).

Results

Apical bud structure

Meristems in both species were dome-shaped structures of similar size (Fig. 2*g, j*) surrounded by newly formed leaves (Fig. 2). The leaves were initiated around the meristem in an alternate (spiral; 2/5) phyllotactic pattern and arranged in ascending order of size, from the newly formed primordium attached to the meristem (Fig. 2*g, j*) to the last folded outer leaf (Fig. 2*a, h*) creating a distinct structure on the top of the shoot. In cucumber plants, the bud contained 22 (±0.36 s.e.; n=8) folded (vertically oriented), lobbed leaves (Fig. 2*a-g*) resulting in 31.7 cm^2 contained leaf area (±2.9 s.e.; n=8).

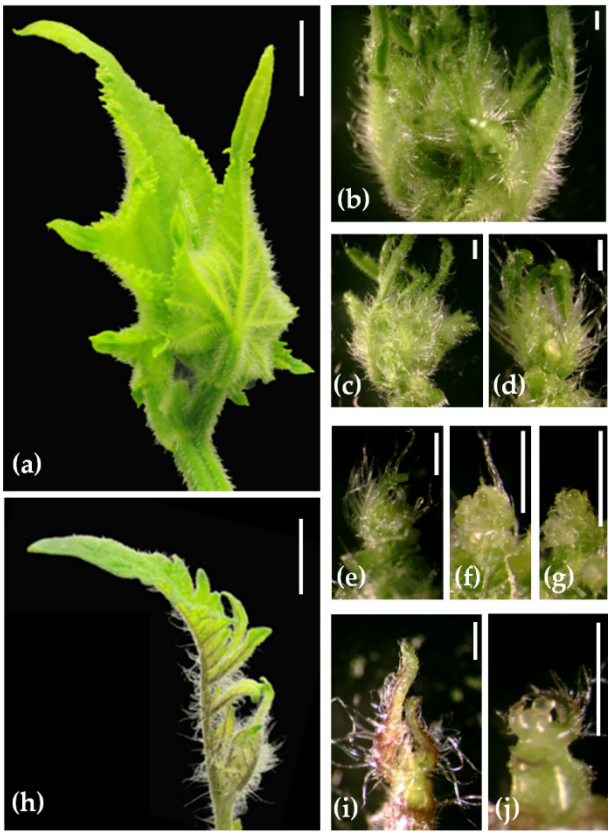

Fig. 2. Apical bud structure in cucumber (*a*) and tomato plants (*h*) after the 7th leaf had unfolded. On the right the apical bud internal structure as observed under the micro-stereoscope by progressively dissecting three leaves in cucumber (*a* to *g*) and in tomato (*h* to j) until reaching the meristem which is surrounded by three leaf primordia (*g* and *j*). Scale bars represent 1cm (*a, h*) or 1mm (*b-g, i-j*).

In tomato plants, the bud contained 9 (±0.17 s.e.; n=8) folded, compound leaves (Fig. 2h-j) resulting in 11.4 cm² contained leaf area (±1.3 s.e.; n=8). Trichomes were present on the leaves comprising the bud in both species. Due to the different contained leaf number and morphology, the bud in cucumber plants was a more voluminous and compact structure while in tomato the bud was more open.

The response of meristem temperature to air temperature

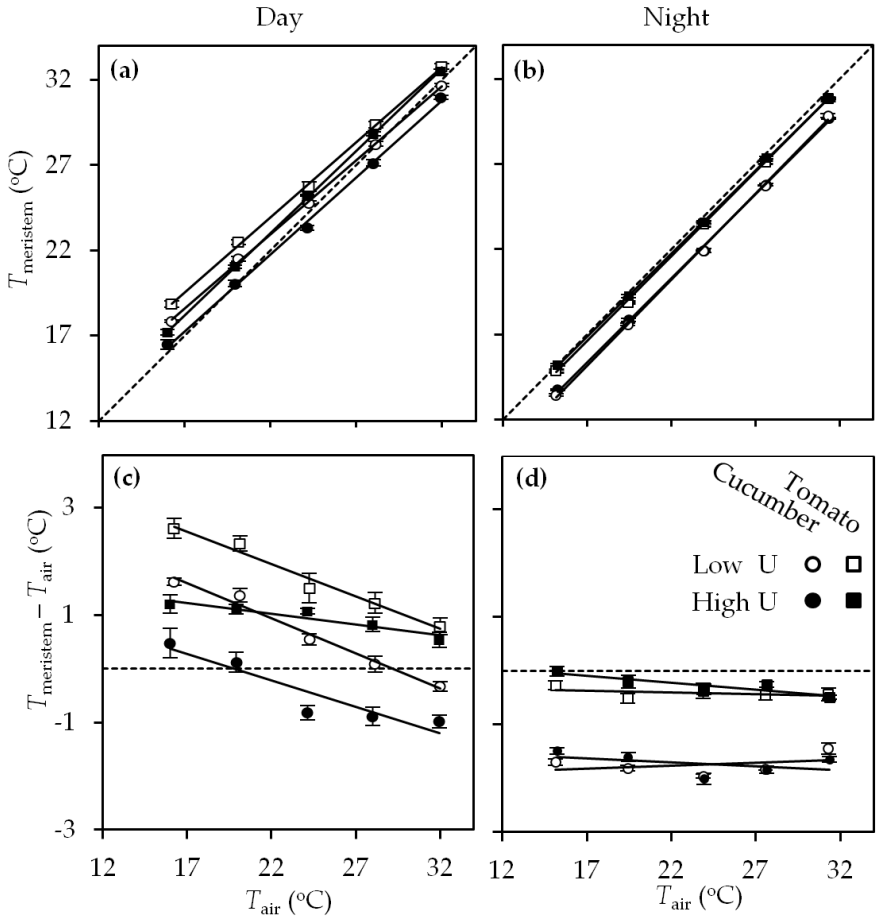

Fig. 3. Meristem temperatures ($T_{meristem}$; *a, b*) and the difference between meristem and air temperatures ($T_{meristem}$ - T_{air}; c, d) during day (left) and night (right) of cucumber (circles) and tomato plants (squares) at low (U = 0.2 m s^{-1}; open symbols) and high wind speed (U = 0.6 m s^{-1}; closed symbols) as a function of air temperature (T_{air}). Values are the means of measurements on 12 plants ± s.e.

In both species, $T_{meristem}$ increased with increasing T_{air} during the day (Fig. 3*a*) and night (Fig. 3*b*). During the day (high R_{net}; Fig. 3*c*), $T_{meristem}$ was higher than at night (R_{net} = 0 W m^{-2}; Fig. 3*d*). High U significantly decreased $T_{meristem}$ during the day (P < 0.001; Fig. 3*c*) but not at night (P = 0.38; Fig. 3*d*). Increasing T_{air} during the day reduced [$T_{meristem}$ - T_{air}] (Fig. 3*c*). This response highly correlated (P < 0.001) with a decreased R_{net} with increasing T_{air} (data not shown). The decrease in R_{net} with increasing T_{air} (16 to 32 ^{o}C) during the day was due to a decrease in R_{LW}, as a result of decreasing difference between $T_{meristem}$ and $T_{ceiling}$ (31 to 36 ^{o}C).

At night, $T_{meristem}$ remained below T_{air} and [$T_{meristem}$ - T_{air}] remained stable with increasing T_{air} (Fig. 3*d*). During the day and night, $T_{meristem}$ in tomato was always higher than in cucumber (Fig. 3*c, d*). At night, $T_{meristem}$ in tomato remained closer to T_{air} ([$T_{meristem}$ - T_{air}] ≈ -0.5 ^{o}C) than in cucumber ([$T_{meristem}$ - T_{air}] ≈ -2 ^{o}C). During the day, an interaction was observed between T_{air} and U on the [$T_{meristem}$ - T_{air}] in tomato (P = 0.002), but not in cucumber (P = 0.13; Fig. 3*c*). In the range of 16-32 ^{o}C T_{air}, the [$T_{meristem}$ - T_{air}] in tomato decreased from 2.6 to 0.8 ^{o}C at low U and from 1.2 to 0.5 ^{o}C at high U. In cucumber, the [$T_{meristem}$ - T_{air}] decreased from 1.6 to -0.3 ^{o}C at low U and from 0.5 to -1.0 ^{o}C at high U (Fig. 3*c*). In tomato, the response of [$T_{meristem}$ - T_{air}] to increasing T_{air} was less steep at high than at low U. $T_{meristem}$ in tomato did not decrease below T_{air}. In cucumber, the response of [$T_{meristem}$ - T_{air}] to T_{air} did not significantly change with U, resulting in negative [$T_{meristem}$ - T_{air}] with increasing T_{air} at high U.

The response of meristem temperature to vapour pressure deficit

$T_{meristem}$ substantially decreased with increasing VPD both during the day (Fig. 4*a*) and night (Fig. 4*b*) in cucumber (P < 0.001), but not in tomato plants (P = 0.99). High U significantly decreased $T_{meristem}$ in both species during the day (Fig. 4*a*); there was no interaction between U and VPD (P = 0.96). At night, high U increased $T_{meristem}$ in tomato towards T_{air}; there was no interaction between U and VPD (P = 0.99; Fig. 4*b*). However, in cucumber high U influenced $T_{meristem}$ only at high VPD during the night; there was an interaction between U and VPD (P < 0.001; Fig. 4*b*).

$T_{meristem}$ in tomato was always higher than in cucumber, both at day (Fig. 4*a*) and night (Fig. 4*b*). The difference in $T_{meristem}$ between the two species increased with increasing VPD. These differences are also reflected on [$T_{meristem}$ - T_{air}]. In the

range of 0.3-1.2 kPa during the day, the difference between $T_{meristem}$ and T_{air} in tomato remained around 2.0 °C at low U and around 1.0 °C at high U. In cucumber, this difference decreased from 1.8 (at 0.3 kPa) to 0.4 °C (at 1.2 kPa) at low U and from 0.6 to -1.3 °C at high U. During the night, the [$T_{meristem}$ - T_{air}] in tomato remained around -0.5 °C at low U and around -0.2 °C at high U. In cucumber, the [$T_{meristem}$ - T_{air}] decreased from -0.7 to -2.9 °C with increasing VPD at low U and from -0.8 to -2.2 °C at high U.

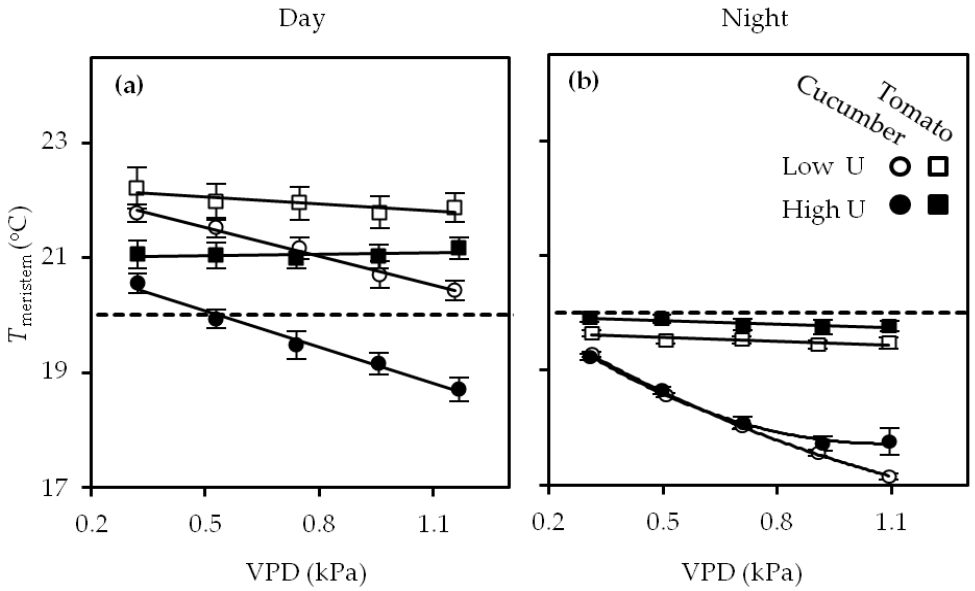

Fig. 4. Meristem temperatures ($T_{meristem}$) during day (*a*) and night (*b*) of cucumber (circles) and tomato plants (squares) at low (U = 0.2 m s^{-1}; open symbols) and high wind speed (U = 0.6 m s^{-1}; closed symbols) as a function of vapour pressure deficit (VPD). Values are the means of measurements on 12 plants ± s.e.

The large interspecific difference observed with increasing VPD was also reflected in thermal images taken on buds at low U and maximum VPD (Fig. 5). During the day, bud temperature in cucumber had an average temperature close to T_{air} while the tomato bud showed higher temperature. At night, bud temperature in cucumber dropped far below T_{air} when compared to tomato. The thermal images were closely related with the measurements performed by thermocouples within the buds (Fig. 4).

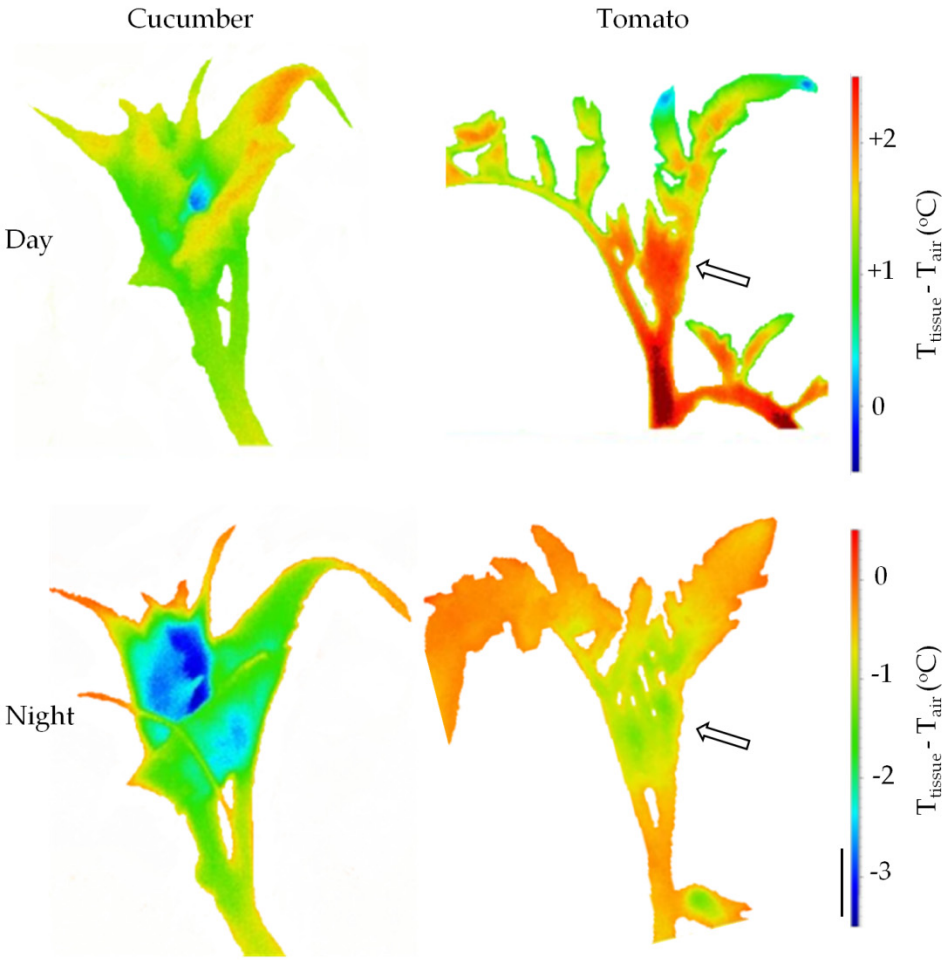

Fig. 5. Thermal images of cucumber (left) and tomato apical buds (right) taken during day (upper) and night (lower) at maximum vapour pressure deficit (VPD = 1.2 kPa) and low wind speed (U = 0.2 m s^{-1}). The arrows indicate the location of the meristem in tomato. Scale bar represents 1cm.

The response of meristem temperature to radiation

$T_{meristem}$ increased with increasing R_{net} in both species (Fig. 6). The response of $T_{meristem}$ to R_{net} was steeper at low U than at high U (interaction between R_{net} and U; P<0.001). High U significantly decreased $T_{meristem}$ during the day and increased $T_{meristem}$ during the night towards T_{air}. Hence, $T_{meristem}$ was always closer to T_{air} at high U than at low U. No differences were observed between species on the response of $T_{meristem}$ to R_{net} (no interaction between species and R_{net}; $P = 0.08$) and U (no interaction between species and U; $P = 0.47$). However, $T_{meristem}$ in tomato was higher than in cucumber plants. These differences were also reflected on [$T_{meristem}$ - T_{air}]. In the range of -80 to 320 W m^{-2}, the [$T_{meristem}$ - T_{air}] in tomato increased from -2.6 to 3.8 °C at low U and from -1.6 to 2.0° C at high U. In cucumber, the [$T_{meristem}$ - T_{air}] increased from -4.1 to 3.0 °C at low U and from -3.5 to 0.9 °C at high U (Fig. 6).

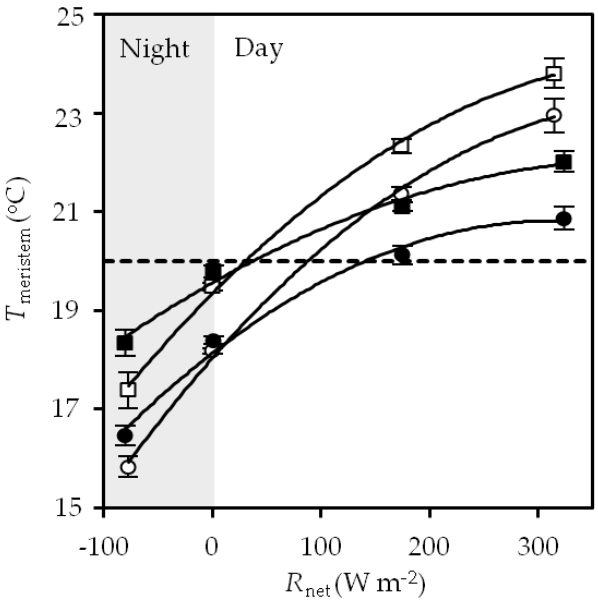

Fig. 6. Meristem temperatures ($T_{meristem}$) of cucumber (circles) and tomato (squares) plants at low (U = 0.2 m s^{-1}; open symbols) and high wind speed (U = 0.6 m s^{-1}; closed symbols) as a function of the net radiation absorbed by a black body (R_{net}). Values are the means of measurements on 12 plants ± s.e.

Apical bud transpiration

E_{bud} increased with increasing $VPD_{bud\text{-}air}$ in both species (Fig. *7a)*. The response of the diurnal E_{bud} to $VPD_{bud\text{-}air}$ was not significantly different from that of the nocturnal E_{bud} ($P = 0.34$; Fig. 7*a*).

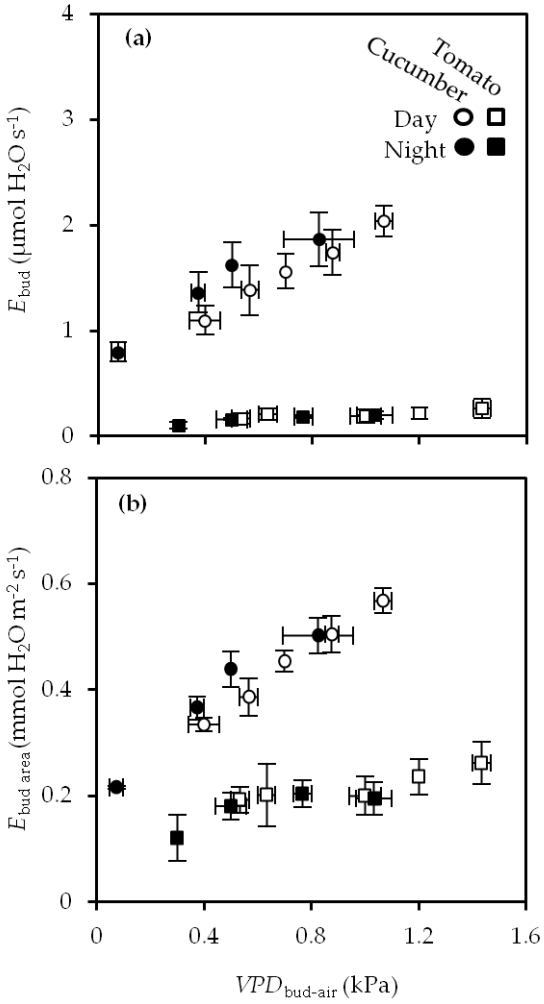

Fig. 7. Transpiration rates of the apical bud per bud (E_{bud}; *a*) and per apical bud-contained leaf area (sum of the adaxial and abaxial leaf surface area; $E_{bud\ area}$; *b*) in cucumber (circles) and tomato (squares) during day (open symbols) and night (closed symbols) as a function of vapour pressure difference between the bud and the air ($VPD_{bud\text{-}air}$). Values are the means of measurements on 4 plants ± s.e.

In cucumber, transpiration rate was significantly higher and showed significantly steeper response to $VPD_{bud\text{-}air}$ ($P < 0.001$) than in tomato ($P = 0.045$; Fig. 7*a*). $E_{bud\ area}$ showed comparable but slightly smaller differences between species due to the 3-fold higher contained leaf area in cucumber in comparison with tomato plants (Fig. 7*b*).

Discussion

Meristem temperature can be substantially higher, or lower than commonly expected

$T_{meristem}$ deviated substantially from T_{air} when plants of both species were subjected to moderate environments. Under the studied environments, [$T_{meristem}$ - T_{air}] in tomato ranged from -2.6 to 3.8 °C, while in cucumber it ranged from -4.1 to 3.0 °C. R_{net}, U, and T_{air} were important determinants of [$T_{meristem}$ - T_{air}] while in cucumber also VPD was an important determinant.

The effects of T_{air} on [$T_{meristem}$ - T_{air}] are complicated because variation of T_{air} in the climate room did not only influence $T_{meristem}$ directly via convective heat transfer between the bud and the air, but also indirectly. It is expected that any increase in T_{air} in the climate room increases $T_{meristem}$ as well as the temperature of the objects in the field of view of the bud (e.g. the ceiling of the climate room). The longwave radiation emitted by a body depends primarily on its temperature (Nobel 2009). Therefore, a similar increase of the temperature of all the objects in the climate room by an increase in T_{air} is not expected to substantially change the longwave radiant heat exchange between the objects in the field of view of the bud and the bud and via that to influence the $T_{meristem}$. This was observed during the night, where $T_{meristem}$ increased equally to T_{air} with increasing T_{air} (Fig. 3*d*). At night, R_{net} was 0 W m^{-2} and the $T_{ceiling}$ strictly followed T_{air}. However, during the day the temperature of the ceiling was higher than T_{air} and an increase in T_{air} did not cause a similar to T_{air}, increase in $T_{ceiling}$ (probably related to the separated cooling of the lamps above the ceiling). This resulted in decreasing R_{LW} with increasing T_{air}. It is expected that a change in the longwave radiant heat exchange between the bud and the surrounding objects (e.g. ceiling) with increasing T_{air} shifts [$T_{meristem}$ - T_{air}].

This suggests that $T_{meristem}$ may not constantly follow T_{air} even when the latter is the only obvious fluctuating environmental variable. Such indirect effects will also occur in greenhouse cultivation and/or experimentation where for example, $T_{ceiling}$ deviates from internal T_{air} due to the influence of ambient environmental factors (e.g. clear night sky).

Extreme environments yield large [$T_{meristem}$ - T_{air}] (Smith 1974; Wilson *et al.* 1987). We here show that even under moderate environments $T_{meristem}$ substantially deviates from T_{air}. Tomato and especially cucumber plants show high LIR and steep responses of LIR to temperature (Marcelis 1993b; Heuvelink 2005). Based on published relations between T_{air} and LIR for tomato (Heuvelink 2005) and cucumber (Marcelis 1994), a small diel deviation of 1 ^{o}C from 20 ^{o}C would substantially alter the LIR by 5% in tomato and 9% in cucumber plants. Likewise, substituting $T_{meristem}$ with T_{air}, potentially results in overlooking the important effects of environmental variables such as U, R_{net}, and VPD on $T_{meristem}$ and via that on LIR. This yields inaccurate study and/or prediction of $T_{meristem}$ effects on LIR.

Apical bud structure and function are key-determinants of meristem temperature

Interestingly, the two species did not experience the same $T_{meristem}$ when subjected to the same environments. The observed differences in $T_{meristem}$ between the two species studied are pointing towards the differences in bud structure and function. The meristems of both studied species were dome-shaped and of similar size (Fig. 2*g, j*) but enclosed in buds, differing in structure (Fig. 2) and function (Fig. 7). Radiant and convective heat exchange as well as latent heat loss are the main components of the heat balance of a plant organ and therefore are important determinants of organ temperature (Jones 1992; Nobel 2009). These heat exchange processes are modulated by the ambient environmental variables but also the thermal traits of the plant organ itself.

Radiant heat exchange is the dominant component of the heat balance (Jones 1992). Quantification of radiant heat exchange in complex structures attracted interest in the past (e.g. conifer branches; Tibbals *et al.* 1964; Gates *et al.* 1965). The vertical orientation of the leaves comprising the bud of both species suggests a reduced absorbed radiation per unit leaf area as compared to unfolded planar leaves, particularly when the main radiant source (for both the shortwave and long wave radiation) is located above the plants (Medina *et al.* 1978). Similarly,

the vertical orientation of bud leaves reduces the net longwave radiant heat loss when subjected to a clear cold night sky (Leuning and Cremer 1988). Despite the previous observations, $T_{meristem}$ was still drastically influenced by varying the radiant environment in both species resulting in large deviations of $T_{meristem}$ from T_{air} (Fig. 6). However, the large effect of the radiant environment on the absolute [$T_{meristem}$ - T_{air}] diminished with increased U as $T_{meristem}$ came closer to T_{air} (Fig. 6). An increase in U is expected to enhance both convective heat exchange (when $T_{meristem} \neq T_{air}$) and latent heat loss (when $VPD_{bud\text{-}air} \neq 0$). This indicates the importance of the convective heat exchange and/or latent heat loss in determining $T_{meristem}$.

The boundary layer is the air layer adjacent to the organ surface and imposes a physical restriction (resistance) to convective heat exchange and latent (by transpiration) heat loss (Schuepp 1993). In organs of simple geometrical profile (e.g. plates, cylinders, spheres), the thickness of the boundary layer (δ_{bl}) and consequently its resistance is associated with the organ shape and size in the direction of air flow and decreases with increasing U (Nobel 1974; Nobel 1975, 2009). In complex structures, δ_{bl} depends on additional traits (e.g. the foliage surrounding the conifer buds; Grace 2006; Michaletz and Johnson 2006). The bud creates a shelter around the meristem and therefore increases the δ_{bl} (Grant 1983). However, the air can easier penetrate a porous than a less porous structure (Landsberg and Thom 1971; Grant 1984). The compact, less porous, and more voluminous character of the bud in cucumber (Fig. 2*a*) implies a higher δ_{bl} than in tomato plants (Fig. 2*h*). The compactness in cucumber buds creates more unstirred, humid, and smaller air spaces moving from the outer folded leaves towards the meristem. Therefore, in cucumber, convective and latent heat exchanges are possibly more constrained by the structure of the apical bud than in tomato plants. In addition, in compact structures like maize ears the conductive heat exchange between the different layers comprising the ear is an important determinant of the organ heat balance and therefore organ temperature (Khabba *et al.* 1999). Similarly, the compact structure of the bud in cucumber probably enables higher conductive heat exchange of the meristem with the surrounding foliage in comparison with tomato plants in which the porous structure enables larger air spaces and high air trespassing between the bud components. Therefore, $T_{meristem}$ in cucumber may be more dependent on the temperature of the surrounding foliage than in tomato.

Latent heat loss is driven by transpiration. The main difference between

the two species studied was the $T_{meristem}$ sensitivity to VPD (Fig. 4) suggesting large differences in latent heat loss. $T_{meristem}$ was almost equal between the species at the lowest VPD. However, increasing VPD resulted in decreasing $T_{meristem}$ in cucumber plants while $T_{meristem}$ in tomato plants was irresponsive (Fig. 4). This study is the first to quantify *in situ* the bud transpiration rates (Fig. 7). E_{bud} positively responded to increasing $VPD_{bud\text{-}air}$ in both species. However, $E_{bud\ area}$ in cucumber was more sensitive to $VPD_{bud\text{-}air}$ and higher than in tomato plants (Fig. 7*b*) indicating a lower resistance to water vapour diffusion through the bud and higher latent heat loss. The meristem in cucumber plants is well sheltered by the outer foliage. The humid microenvironment of the meristem created by the outer foliage suggests low to negligible transpiration by the meristem itself and the lastly formed primordial leaves. Therefore, the large sensitivity of $T_{meristem}$ to the VPD can only be related to the transpiration of the outer foliage of the apical bud that is subjected to less humid environment and the direct cooling of meristem by conduction with the outer foliage.

In both species the response of E_{bud} to $VPD_{bud\text{-}air}$ was not significantly different between day and night (Fig. 7). This explains why during the night (R_{net} = 0 W m^{-2}) $T_{meristem}$ was lower than T_{air} in both species (Fig. 3*d*, 4*b*). The existence of nocturnal E_{bud} was already suggested in previous studies (Faust and Heins 1998) though it has never been measured before. The equality between the diurnal and nocturnal relationship of E_{bud} with $VPD_{bud\text{-}air}$ suggests that the E_{bud} of the studied species is not physiologically controlled by stomatal responses to environmental stimuli. Indeed, in very young aerial tissues the water vapour diffusion through the epidermis cannot be solely controlled by stomatal function due to the high cuticle permeability (underdeveloped cuticle) to water vapour (Hauke and Schreiber 1998; Richardson *et al.* 2007) and the high number of occluded and underdeveloped stomata (Snider *et al.* 2009).

Small or even large visible structural and 'invisible' functional differences between plant species are usually neglected especially for plant organs other than planar leaves. The interspecific differences in structure and transpiration of the buds observed here were more than enough to cause substantial differences in $T_{meristem}$ between the two plant species studied. Consequently, meristem temperatures should be treated as species-specific traits highly reliant on the environment.

Implications in the study of plant ecophysiology and future directions

The necessity of coupling the biological processes taking place within a plant organ with the physical environment actually perceived by the organ (phylloclimate; Chelle 2005) is well illustrated by our results. $T_{meristem}$, rather than T_{air}, is the link between the environment and plant development. In this study, we have shown that even under controlled environments, usually used in experimental practice (i.e. climate rooms), $T_{meristem}$ may largely deviate from T_{air} as well as between species. Therefore, $T_{meristem}$ quantification should be incorporated in the 'to-do' list for proper experimentation (Poorter *et al.* 2012), especially when investigating the sensitivity of plant developmental processes to temperature (Parent and Tardieu 2012). $T_{meristem}$ quantification is needed to properly distinguish between the factors influencing LIR through effects on $T_{meristem}$ and factors influencing LIR directly (e.g. daylength; Jamieson *et al.* 1995; e.g. radiation; Trouwborst *et al.* 2010). Therefore, $T_{meristem}$ quantification is needed to properly study the plant-environment interactions. Likewise, the incorporation of $T_{meristem}$ instead of T_{air} in plant growth models would certainly yield more precise predictions of plant development.

We have also shown that bud structure and function are important determinants of $T_{meristem}$. Bud structure and function, and therefore, $T_{meristem}$ greatly vary between species. Even between different genotypes of the same species differences in $T_{meristem}$ can appear. Apple and co-authors (1999) suggested that small alterations in the structure of the bud of Douglas-fir may be crucial for $T_{meristem}$. Bud structure and function similarly to leaf structure (Hanson 1917) and function (Hetherington and Woodward 2003), may be also subject to adaptation in environmental fluctuations. Additionally, buds are dynamic structures; they may structurally and functionally shift in the course of plant development. Overall, $T_{meristem}$ should be considered as a plant trait varying in spatiotemporal scale that is worth quantification also in phenotyping and breeding programs.

The astonishing cooling capacity of the bud in cucumber plants and, on the other hand, the insensitivity of $T_{meristem}$ to VPD in tomato plants suggests further investigation of the structure and function when attempting modelling and more precise prediction of $T_{meristem}$. It is not sufficient to assume that plant structures can be represented by simple geometric objects (Grace 2006). Due to the discrete structural and functional traits of the apical bud shown in this study (when compared to planar leaves), meristems may perceive different temperatures

than planar leaves. Therefore, planar leaf temperature cannot be securely considered as an approximation of the plant temperature as a whole. In addition, organ heat balance may be well influenced by intra-canopy heat exchange (Guilioni and Lhomme 2006). Therefore, meristem, or in general organ temperature and its determinants may be optimally realized when considering heat exchange at more than one level of organization (e.g. organ, plant, population) in the future. We propose the incorporation of $T_{meristem}$ and its determinants in the emerging field of structural-functional plant models (Chelle 2005; Kahlen and Stützel 2011; Sarlikioti *et al.* 2011).

Climate change has already triggered substantial shifts in species distribution and abundance (Walther *et al.* 2002). Climate change scenarios predict even more detrimental effects leading to massive species extinction (Thuiller *et al.* 2005) as well as severe damages to crop yields (Schlenker and Roberts 2009). Predictions on the effects of climate change are mostly based on environmental variables like T_{air}. However, previous studies indicate that the patterns of organismal stress can be more precisely predicted based on body temperature (Broitman *et al.* 2009; Helmuth *et al.* 2010). The substantial deviation of $T_{meristem}$ from T_{air} indicated in this study, even under moderate environments, strengthens the necessity of incorporating plant organ temperatures to properly predict the effect of climate change on plant growth. On top of that, the species-specific response of $T_{meristem}$ to the environment suggests species-specific responses to climate change even when the species are sharing the same habitat.

We here indicate that for properly linking growth and development of plants to temperature in future applications, for instance in climate change scenarios studies, $T_{meristem}$ should be used instead of T_{air}, as a species-specific trait highly reliant on various environmental factors.

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Chapter 3

Leaf initiation is solely dependent on the apical bud temperature even under large bud-plant temperature differences

Abstract

Leaf initiation is a critical process for plant growth (models) and its rate (LIR) is very sensitive to temperature. In most models, relating plant development to the environment, not the temperature of the apical bud (T_{bud}), but the ambient- (T_{air}) or a general plant-temperature is used to calculate LIR. In many natural and agricultural environments, T_{bud} may significantly differ from T_{air} or the temperature of the rest of the plant (T_{plant}). If T_{bud} solely influences LIR, its poor approximation will lead to serious misinterpretation of experimental results and miscalculations in models. If beside T_{bud}, T_{plant} also influences LIR (through systemic signals), predictions will become even more problematical. We investigated whether LIR solely depends on T_{bud} when T_{bud} is independently altered from T_{plant}. Using a custom-made device, T_{bud} was altered in cucumber plants yielding 9 combinations of T_{bud}/T_{plant} between 18-26 ^{o}C and LIR was quantified. LIR increased by 12% per ^{o}C of T_{bud} regardless T_{plant}. The sole response of LIR to T_{bud}, even under major intra-plant temperature differences, implies a strong and singular relation between bud function and local temperature perception. Consequently, accurate measurements or realistic estimates of T_{bud} should be used in experimental and modelling studies in which plant development is a key issue.

Savvides A, Dieleman JA, van Ieperen W, Marcelis LFM, submitted for publication.

Introduction

Most plant species do not actively control their temperature and therefore cannot maintain thermal homeostasis. Plant temperature fluctuates depending on the environment (Jones 1992). Therefore, plants are 'classified' as poikilotherms (McNaughton 1972; Körner 2006). Misinterpretation of this term probably triggered the to-date common assumption that plant temperature strictly follows air temperature (T_{air}). The fact that plants cannot actively control their own temperature does not necessarily imply that the plant temperature follows T_{air}. Other environmental factors (e.g. radiation, wind speed, humidity) in interaction with heat transfer-related plant traits (e.g. transpiration capacity, morphology) can also modulate the heat budget of a plant. As a consequence plant temperature may considerably deviate from T_{air}. For instance, meristem temperature was found to deviate from T_{air} from -2.6 to 3.8 ^{o}C in tomato and from -4.1 to 3 ^{o}C in cucumber plants under moderate environments (Savvides *et al.* 2013).

Plant temperature is not always uniform either. Vertical intra-plant temperature differences, mainly caused by vertical microclimatic differences, were observed in nature (Gibbs and Patten 1970), field crop cultivation (Gardner *et al.* 1981) and in protected cultivation (Kempkes and van de Braak 2000; Li *et al.* 2014). In contrast to other plant microclimate heterogeneities (e.g. light gradients; Pons *et al.* 2001), the effects of such temperature heterogeneities on plant development have been hardly studied.

Temperature highly and predictably influences phytomer (i.e. shoot modules usually comprised of an internode, a leaf and an axillary bud) or leaf initiation rates (Granier and Tardieu 1998; Granier *et al.* 2002). Phytomer initiation rate, or most commonly, leaf initiation rate (LIR) defines the number of phytomeres on a plant per unit of time and therefore determines the shoot longitudinal growth and plant architecture. Consequently, LIR is an important plant trait used in a wide range of plant growth models (e.g. Marcelis *et al.* 1998; Vos *et al.* 2010; Pallas *et al.* 2011; Zhu *et al.* 2014).

Leaf initiation takes place on the shoot apical meristem (SAM). The SAM is a group of undifferentiated cells usually hidden within young folded developing leaves forming the apical bud, a distinct structure on the top of the shoot. The top

of the shoot may be subjected to different solar radiation during the day (Gibbs and Patten 1970), wind speeds (Tuzet *et al.* 1997) and/or nocturnal terrestrial (sky and soil) thermal radiation (Leuning and Cremer 1988) than the lower part of the shoot due to the higher exposure of the top shoot to the extra-canopy environment. Therefore, apical bud temperature (T_{bud}) may considerably deviate from the temperature of the rest plant (T_{plant}).

Previous studies suggested that it is more accurate to link LIR to apical bud (or shoot apex) temperature instead of T_{air} (Jamieson *et al.* 1995; Granier and Tardieu 1998). To the best of our knowledge, there is no experimental evidence proving that LIR is not also influenced by plant temperatures other than T_{bud}. In several cases, environmental cues (e.g. temperature, light intensity, ambient CO_2 concentration) are sensed by the mature plant tissues (e.g. leaves) and systemic signals from these tissues are mediating developmental changes in young tissues (Lake *et al.* 2001; Coupe *et al.* 2006; Gorsuch *et al.* 2010). These systemic signals are potentially acting as a warning system to enable young tissues to cope with their current environment (Gorsuch *et al.* 2010). It is also worth mentioning that LIR may be highly influenced by low light intensity (Savvides *et al.* 2014), increased number of sinks (Marcelis 1993b) or leaf (source) removal (Hussey 1963b) suggesting a systemic control of LIR. This strengthens the hypothesis that LIR may not only be influenced by the local perception of the environment (e.g. T_{bud}). It is then well possible that LIR may also be influenced by plant temperatures other than T_{bud}.

The aim of this study was to investigate whether LIR is only modulated by T_{bud} regardless T_{plant}. For this, we developed a custom made heating/cooling system in which the temperature of the apical bud could be manipulated while maintaining the temperature of the rest of the plant at another level. *Cucumis sativus* plants were used in this study as they are fast-growing plants of indeterminate growth.

Materials and methods

Plant material and growth conditions

Cucumber (*Cucumis sativus* cv. Venice RZ) plants were grown in a climate room at 22 °C T_{air}, 70% relative humidity (RH; VPD = 0.8 kPa) and ~380 μmol mol^{-1} [CO_2] on rockwool slabs and watered with nutrient solution (EC = 2 dS m^{-1}, pH = 5.0 - 5.5). The plants were illuminated by SON-T lamps (MASTER GreenPower CGT 400W E40 1SL; Royal Philips Electronics N.V., Amsterdam, The Netherlands) at a photosynthetic photon flux density (PPFD) of 200 μmol m^{-2} s^{-1} during 16 h photoperiod. Two lamps were installed per m^2 to achieve homogeneous distribution of light intensity. The lamps were isolated from the climate cell by a glass ceiling which enabled the separate convective cooling of the lamps and an energy screen (OLS60; *AB Ludvig Svensson*, Kinna, Sweden) was added below the glass ceiling to reduce the thermal radiation emission by the lamps and maintain the homogeneous distribution of light intensity in the climate room. After the 7th leaf had unfolded (~28 days after plant emergence) and the apical buds were distinct structures on the top of the plant canopy (prior this stage apical buds are hidden below the youngest unfolded leaves), plants were subjected to various bud – plant temperature differences (T_{bud}-T_{plant}).

Table 1. Plants were treated with nine different combinations of apical bud temperature (T_{bud}) and plant temperature (T_{plant}) resulting in different T_{bud} - T_{plant} in the range of -8 to +8 °C.

Treatment (T_{bud}/T_{plant})	T_{bud} (°C)	T_{plant} (°C)	T_{bud}-T_{plant} (°C)
18/18	18	18	0
22/18	22	18	+ 4
26/18	26	18	+ 8
18/22	18	22	- 4
22/22	22	22	0
26/22	26	22	+ 4
18/26	18	26	- 8
22/26	22	26	- 4
26/26	26	26	0

Temperature treatments

Plants were subjected to 9 different combinations of T_{bud}/T_{plant} in the range of 18-26°C (18/18, 22/18, 26/18, 18/22, 22/22, 26/22, 18/26, 22/26, 26/26; Table 1). The differences between T_{bud} and T_{plant} (Table 1) were achieved by maintaining T_{bud} at 18, 22 or 26 °C using a custom-made device (see below) and maintaining T_{plant} (the temperature of the rest of the plant) by controlling T_{air} in three treatments differing in T_{air} (18, 22 and 26 °C) that were carried out one after the other. Eight plants were subjected to each T_{bud}/T_{plant} combination for 28 days. During plant development side shoots were removed when at maximal 2 cm length. In all treatments, fruits were only allowed to develop at every 4th internode starting from the 10th internode to avoid uneven fruit set and abortion and thereby to keep the photosynthate allocation balanced.

Apical bud heating/cooling system

T_{bud} was altered and maintained stable during the treatments by convective heating/cooling (i.e. changing air temperature locally) using a custom-made heating/cooling (h/c) system (Fig. 1). The VPD and wind speed local to the bud were also controlled as they are also largely influencing T_{bud} in cucumber plants (Savvides *et al.* 2013).

Temperature regulation: After the 7th leaf had unfolded, the apical bud was carefully enclosed within a transparent hollow PVC sphere (8 cm internal diameter; Fig.1b) to enable the local control of T_{bud}. The sphere was comprised of two hemispheres. The lower junction point (a circle, 3 cm diameter) of the two hemispheres was removed and replaced by two semi-circular pieces of silicon rubber lamina (3 mm thick) to prevent stem damage (Fig.1b). The sphere allowed ~90% light transmittance without affecting the light spectrum. To avoid light intensity differences (at apical bud level) between the treatments, all the plants were enclosed in spheres and their T_{bud} was controlled by the h/c system.

Each sphere was supplied with (humidified) air of certain temperature (18-26 °C). The air was heated/cooled and its temperature was maintained by an h/c device (Fig. 1a, c). The treated air was transported from the h/c device to the sphere via a polyethylene (PE) tube (7 mm internal diameter). One h/c module (i.e. the combination of a sphere and an h/c device) was used per plant (Fig. 1c) enabling the separate control of T_{bud} per plant. The PE tube was insulated to maintain the

temperature of the air despite the ambient conditions by being inserted into elastomeric thermal insulation (FR/Armaflex®, Armacell Enterprise GmbH & Co. KG, Münster, Germany), which was in turn covered with highly reflective material (Fig. 1b). The h/c device was primarily an acrylic chamber via which the compressed air was passing through. Through its passage, the air was heated by a

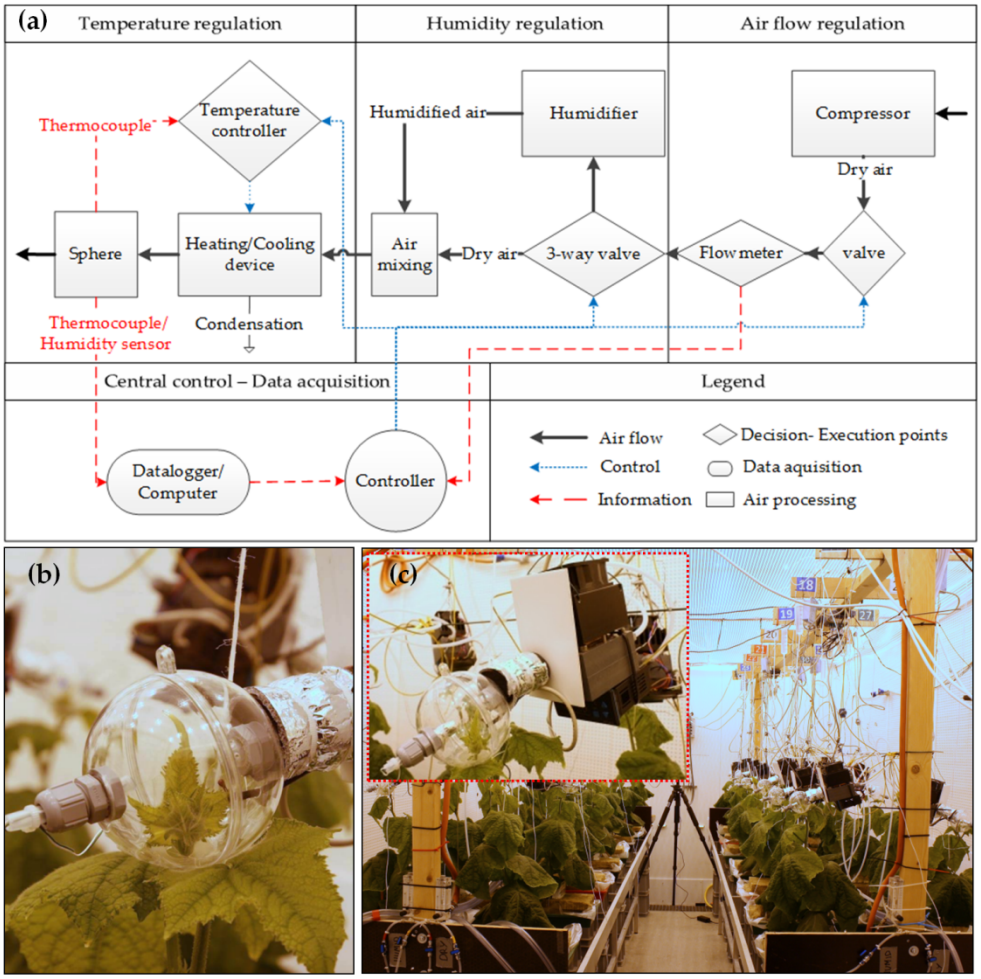

Fig. 1. Schematic representation of the heating/cooling system used to alter apical bud temperature in cucumber plants (a). Transparent sphere used to isolate apical bud from the ambient environment (b). Experimental setup in the climate room and a magnified image of one of the heating/cooling modules attached on a cucumber plant (c).

heating element or cooled by a Peltier element both built in the chamber and controlled in an on/off mode by a temperature controller (ET1412 digital thermostat, ENDA, Istanbul, Turkey) located below the h/c chamber (Fig. 1c). The Peltier element was positioned on the top of the h/c chamber (Fig. 1c). Its cooling surface was enclosed within the chamber while its heated (outer) surface was attached to a heat sink which was in turn attached to a computer fan enabling the heat dispersal from the Peltier element to the ambient and consequently its maximum capacity and proper function. A white plastic surface was used to prohibit the warm air deriving from the heat sink to influence the sphere temperature (Fig. 1c). Sphere temperature (internal air temperature) was communicated to the temperature controller by a thermocouple (t/c) inserted into the sphere (Fig. 1a) and covered with highly reflective material (Fig. 1b) to avoid direct heating of the t/c by radiation. This allowed the precise regulation and maintenance of the air temperature inside the sphere. The air temperature inside the sphere was continuously monitored (every minute) by another t/c connected to a data logger (USB TC-08, Pico Technology, Cambridgeshire, UK) and temperature data were acquired by a computer (Fig. 1a). A minor opening (in comparison to the diameter of the PE tubing system) below the heating/cooling chamber enabled the depletion of condensation from the chamber produced when the humidified air was suddenly cooled down inside the h/c chamber. The 24 h/c devices used were electrified by three power supply units (SPS 9400, Maas, Elsdorf - Berrendorf**,** Germany).

The h/c modules should be able to follow the upward movement of the apical buds due to shoot elongation in time. Therefore, the h/c modules were held via wires on a wooden stand on the top of the plants (Fig. 1c) which enabled their individual vertical movement. Young phytomeres with almost unfolded leaves were carefully removed from the sphere by removing the one (removable) of the two hemispheres and simultaneously moving the h/c module upwards. H/c module adjustments were taking place twice a day (early in the morning and late in the afternoon).

Vapour pressure deficit regulation: To maintain the same VPD (~0.8kPa) in all treatments, the dry compressed air inserted to the h/c system was appropriately humidified prior the entrance in the h/c device. Eight h/c modules per treatment were in parallel connected through PE tubing to a humidifying system to enable

the different adjustment of VPD in the sphere for different T_{bud}. In total 24 h/c modules and 3 humidifiers were used per T_{air} treatment. A fraction of the compressed dry air was bypassed through a humidifier. The fraction was controlled manually by a three-way valve (Fig. 1a). The humidifier was a sealed barrel (50 l) half-filled with de-ionized water (to avoid salt accumulation in the h/c system) via which the air was forced to pass by submerging the cut end of the dry air-bearing PE tube. After humidification, the compressed humidified air was directed via another non-submerged PE tube outside the barrel and mixed in the way to the h/c device using a T-tubing connection with the volume of dry air that bypassed the humidifier. Relative air humidity in the sphere was continuously monitored by a humidity sensor (WS - DLTc, Wireless Value, The Netherlands) and the data were collected to a computer (Fig. 1a). VPD was then calculated based on relative humidity and air temperature inside the sphere.

Wind speed regulation: Wind speed in the sphere was maintained at the levels of the ambient wind speed (~0.2 m s^{-1}) by controlling the air flow prior the humidification of the compressed dry air (Fig. 1a). Air flow was continuously monitored by an air flow-meter (ENK5FRH, Kutola Instruments, Muurame, Finland) and controlled manually using a valve connected on the PE tubing system before the flow-meter in the direction of flow (Fig. 1a). Ambient and sphere wind speed were measured by a 3d-anemometer (WindMaster™; Gill Instruments LTD, Hampshire, UK) and an air velocity meter (Velocicalc 8347, TSI, MN, USA) respectively prior the treatments.

Plant temperature measurements

In a pilot experiment prior the treatments we tested 1) whether the air temperature inside the sphere was a good proxy of the actual T_{bud} and 2) whether T_{plant} was uniform and comparable to T_{air}. T_{bud} (measured by gently inserting K-type t/c into the centre of the bud) strictly followed the air temperature inside the sphere when air temperature inside the sphere was set at 18, 22 and 26 °C at 21.5 °C ambient temperature (Fig. S1a). Therefore, the air temperature in the sphere was securely considered as T_{bud} to avoid damaging the meristematic tissues by direct bud temperature measurements during the treatments. The temperatures of the apical bud, 9th leaf (mid shoot) and 5th leaf (bottom shoot) were measured (leaf temperatures were measured by t/c attachment on the abaxial side of the leaf

lamina) when the 15th leaf had been unfolded on cucumber plants not bearing spheres. The temperatures of the organs measured were similar to T_{air} (Fig. S1b). Therefore, T_{air} was securely considered as T_{plant}.

Leaf initiation rate

LIR was defined as the number of leaves initiated during the treatments divided by the treatment duration of 28 days. The number of leaves initiated during the treatments was obtained by counting the total number of leaves on plants at the start (destructive measurements on 8 representative plants per treatment) and the end of the treatments (destructive measurements on 8 plants per treatment). The visible (to the naked eye) leaves were counted by eye while the very young and invisible leaves (leaf primordia) in the apical bud were quantified by dissecting the apical bud under a stereomicroscope (Wild M7 S, Heerbrugg, Switzerland; 60x – 310x). The latest initiated leaf primordium was defined as the latest formed projection that was visible at the side of the meristem (dome). The number of leaves initiated per unit thermal time (degree (°C) - days) was defined as LIR_{dd} and was based on T_{bud}. Thermal time (in degree [°C] - days) was estimated based on:

$$Thermal\ time = \sum_{n=1}^{k}[(T_{bud})_n - T_{base}] \qquad \textbf{(Eqn. 1)}$$

T_{bud} is the diel integration (mean) of bud temperature while T_{base} is the base temperature at which cessation of the developmental process occurs (Trudgill *et al.* 2005). k is the duration of the treatments in days. The T_{base} used was 10°C and it was estimated by plotting the LIR against T_{bud} and by projecting the resulted linear relationship backwards until null LIR (Fig. 2a).

Statistical analysis

The statistical analysis was performed using SPSS statistics v22.0 for Windows (SPSS IBM, NY, USA). One-way analysis of variance (ANOVA) was used and statistically significant differences on T_{bud}, VPD in the sphere and LIR_{dd} between treatments' means were evaluated with *post hoc* Tukey's honestly significant (HSD) multiple comparison tests ($P < 0.05$). General linear model was fitted to the data to test for statistical significance ($P < 0.05$) of the effects of T_{bud}, T_{plant} and their interaction (T_{bud} x T_{plant}) on LIR.

Results

The response of leaf initiation to apical bud temperature

LIR was statistically significantly affected by T_{bud} ($P < 0.001$) and was not significantly affected by T_{plant} ($P = 0.07$). LIR increased linearly with T_{bud} at a rate of 12.1 % per °C in the range of 18-26 °C (Fig. 2a). LIR was not influenced by T_{plant} or the magnitude of T_{bud}-T_{plant} (Fig. 2b).

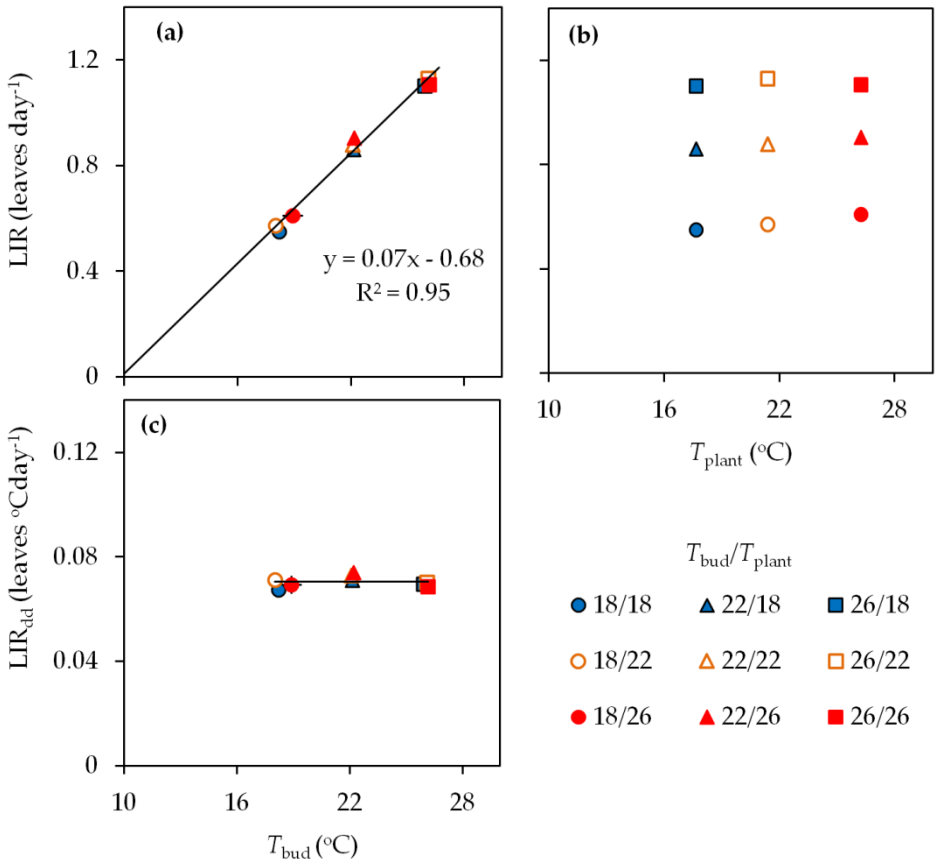

Fig. 2 Leaf initiation rate (LIR; n=8) increased linearly (12.1% per °C) with apical bud temperature (T_{bud}) in the range of 18-26 °C (a) regardless the variations in the temperature of the rest of the plant (T_{plant}; b). LIR normalized with T_{bud}-based thermal time (LIR_{dd}) did not differ across the treatments (c). Values are the means of measurements on 8 plants ± s.e.

Backward projection of the linear relation between LIR and T_{bud} until zero LIR, suggested a base temperature (T_{base}) of 10 °C (Fig. 2a). When LIR was normalized with T_{bud}-based thermal time (LIR_{dd}) it was not significantly different across treatments ($P = 0.09$; Fig. 2c).

Apical bud heating/cooling system performance

T_{bud} was effectively controlled by the custom-made apical bud heating/cooling system in short- (pilot experiment; Fig. S1a) and long-term (main experiments; Table 2). The VPD in the sphere was kept in the range of 0.6 – 0.9 kPa across treatments (Table 2). Statistically significant differences regarding VPD in the sphere were observed between some treatments but these differences were not systematic (Table 2). As a result, LIR was not statistically significantly influenced by VPD ($P = 0.24$) when VPD was included as a factor in the general linear model.

Table 2. Plant temperature (T_{plant}) and vapour pressure deficit (VPD) in the ambient prior the treatments and during the treatments, apical bud (sphere) temperature (T_{bud}; n=8), VPD in the sphere (n=4) and apical bud-based thermal time (n=8) over the treatments. Different letters within a column indicate significant differences ($P < 0.05$).

Treatment (T_{bud}/T_{plant})	Before treatments		During treatments				
	Ambient		Ambient		Sphere		
	T_{plant} (°C)	VPD (kPa)	T_{plant} (°C)	VPD (kPa)	T_{bud} (°C)	VPD (kPa)	Thermal time (°C days)
18/18					18.2^{cd}	0.89^{a}	229^{cd}
22/18	22.0	0.81	17.7	0.77	22.1^{b}	0.82^{ab}	339^{b}
26/18					25.9^{a}	0.75^{ab}	444^{a}
18/22					18.0^{d}	0.65^{b}	225^{d}
22/22	22.1	0.82	21.4	0.70	22.1^{b}	0.63^{b}	338^{b}
26/22					26.1^{a}	0.77^{ab}	451^{a}
18/26					18.9^{c}	0.86^{a}	249^{c}
22/26	22.2	0.80	26.2	0.74	22.2^{b}	0.87^{a}	341^{b}
26/26					26.2^{a}	0.79^{ab}	452^{a}

Discussion

Leaf initiation rate is driven only by the apical bud temperature

Leaf initiation rate follows apical bud temperature (Fig. 2a) even when bud temperature largely deviates from the temperature of the rest of the plant (e.g. T_{bud} - T_{plant} = ±8 ^{o}C; Table 1) in *Cucumis sativus*. This reveals the sole localized (to the apical bud) temperature perception and consequently the independent (from the rest of the plant) response of the apical bud to temperature regarding LIR. The present findings add to a better understanding of plant developmental responses to a spatially diverse environment and promote the implementation of this knowledge in future studies and applications.

Phenotypic plasticity is the ability of plants to adaptively alter morphological, anatomical, or physiological functional traits to local environmental variations using external environmental cues (Niklas 2009). The local perception of temperature by the apical bud is in accordance with the view of De Kroon et al. (2005) that phenotypic plasticity is usually expressed at a sub-individual level. In other words, phenotypic plasticity is a property of individual plant organs, modules or segments triggered by local environmental conditions (De Kroon *et al.* 2005). This allows plants to better exploit the spatial-temporal variation in environmental conditions such as the availability of light (Evans and Cain 1995) and light quality (Thompson 1993), water (Bell and Sultan 1999) and nutrients (Robinson 1994). This study, one of the few focussing on spatial plant temperature variations, reveals that the apical bud function is responsive only to the local temperature regarding LIR. Consequently, the number of phytomeres initiated per unit of time is determined by T_{bud}. Plants are known for their 'incapability' to 'walk away' from unfavourable environments. However, they are also known for their capability to endure and grow in these environments using certain unique tricks. The apical bud can be considered as the plant's growing point which can facilitate plant's 'movement' towards certain directions by the addition of new phytomeres on the longitudinal shoot axis. Consequently, the rate in which these phytomeres are initiated indicates the extent in which plants invest towards a certain direction per unit of time. It is then reasonable to speculate that plants are making use of the localized temperature perception in their apical buds

to track and use spaces with more favourable environments. For example a crawling or climbing *Cucumis sativus* plant would highly benefit by 'moving' from an environment promoting lower to an environment promoting higher (more optimum) plant temperature. This 'movement' can be achieved by the faster outgrowth of apical buds (i.e. higher LIR) that are located at sites of a plant promoting more favourable T_{bud} (e.g. higher radiation in combination with lower wind speeds; Savvides *et al.* 2013) and slower outgrowth of (i.e. lower LIR) apical buds that are located at sites promoting less favourable T_{bud}.

Even if phenotypic plasticity is expressed at a sub-individual level, communication and behavioural integration of interconnected modules can change the local responses (De Kroon *et al.* 2005). For example, LIR can also be influenced by factors like photosynthate (resource) availability (Hussey 1963b; Marcelis 1993b; Savvides *et al.* 2014). Therefore, a plant will not immoderately invest in producing new phytomeres with higher rates when at low resource availability. It is however astonishing that even on the long term (weeks) LIR was the same at plant temperature of 18 and 26˚C, as long as T_{bud} was the same in both treatments. In other words, plants with equal T_{plant} showed different phytomer numbers per unit of time depending on T_{bud}. If a plant is prioritizing its investments towards the longitudinal axis of shoot growth when T_{bud} is higher than T_{plant} and not when at the opposite situation how will plant morphology and growth be influenced under these intra-plant temperature differences? Füllner *et al.* (2012), in one of the few studies focussed on the effects of spatial plant temperature differences, have shown that vertical gradients in root temperature stimulated plant development and increased biomass accumulation in barley. Investigation of the plant responses to spatial plant temperature differences is still a mystery in plant ecophysiology worth to be further unravelled if we want to properly link plant growth to the environment that is actually perceived by plants.

Implications and future directions in the study of plant ecophysiology and related applications

The localized perception of temperature regarding LIR validates and improves assumptions made by several studies that local tissue temperature should be quantified, modelled, predicted and used in relation with plant development (e.g.

Guilioni *et al.* 2000; Vinocur and Ritchie 2001; Chelle 2005; Grace 2006; Craufurd and Wheeler 2009; Savvides *et al.* 2013).

We here show the necessity of coupling developmental processes, like leaf initiation, taking place in the apical bud with the temperature actually perceived by this organ in plant growth models. This can be progressively achieved by firstly downscaling to plant organ (instead of canopy) microclimate modelling (Chelle 2005) and by secondly integrating to plant level by coupling organ microclimate models with functional structural plant models (Vos *et al.* 2010). Up-scaling, coupling phenological models to climate change scenarios (Kramer *et al.* 2000) is of great importance for estimating the responses of plant communities to global warming in the future. However, predictions on the effects of climate change are mostly based on environmental variables like T_{air}. If we are to properly estimate the effects of climate change on plant growth it is not enough to assume that plant physiology is driven by T_{air} (Grace 2006). Consequently, coupling plant organ microclimate models with current phenological models will add to the preciseness of climate change scenarios studies.

Conclusions

Leaf initiation rates follow apical bud temperature even under large bud-plant temperature differences. The sole response of LIR to T_{bud}, even under major intra-plant temperature differences, implies a strong and singular relation between bud function and local temperature perception. Consequently, accurate measurements or realistic estimates of T_{bud} should be used in experimental and modelling studies in which plant development is a key issue.

Acknowledgments

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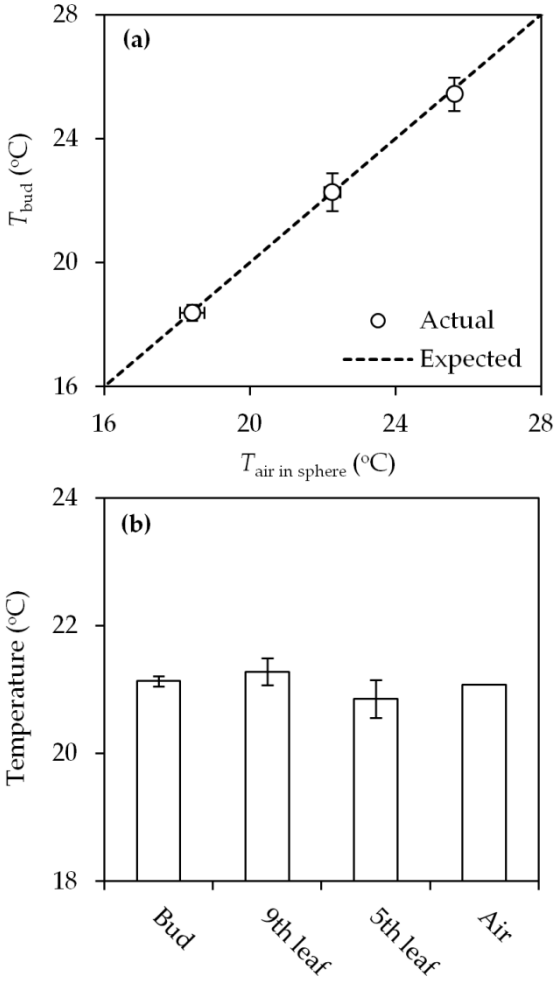
Appendix

Fig. S1. Apical bud temperature (T_{bud}) as a function of the air temperature inside the sphere ($T_{air\ in\ sphere}$; n=4; a) and temperatures of the apical bud (n=4), 9th leaf (middle shoot; n=4) and 5th leaf (bottom shoot; n=4) and air temperature (b) during the pilot experiment.



Chapter 4

Phenotypic plasticity to altered apical bud temperature: more leaves-smaller leaves and vice versa

Abstract

Leaf initiation and subsequent leaf expansion were suggested to be well-coordinated processes with plant temperature resulting in well-defined plant phenotypes. Though, plant temperature may be spatially heterogeneous. Apical bud, the structure in which the initiation and initial growth of new leaves/phytomeres takes place, is located on the top of the shoot which is more subjected to the extra-canopy environment than the lower shoot. Hence, bud temperature (T_{bud}) may largely deviate from the temperature of the rest of the plant (T_{plant}). Recent research showed that T_{bud} influences leaf initiation independent of T_{plant}. Though, the effects of altered T_{bud} on leaf expansion and whole plant phenotype remained to be unravelled. Using a custom-made device, T_{bud} was altered in cucumber plants yielding 9 combinations of T_{bud}/T_{plant} between 18-26 ^{o}C. Increasing T_{bud} beyond T_{plant} resulted in more and smaller leaves while decreasing T_{bud} below T_{plant} resulted in less and larger leaves. This offset between leaf number and individual leaf area indicates a strict systemic coordination between leaf initiation and leaf expansion. The same patterns as for leaf area distribution were observed for biomass distribution across phytomeres. Cucumber plants adjust their phenotype to increased or decreased T_{bud} by reallocating their investments into more or less phytomeres respectively.

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Introduction

Phenotypic plasticity is the ability of plants to adapt their morphology, anatomy and physiology to changes in their direct environment (Niklas 2009). Phenotypic plasticity in response to temperature was extensively studied in leaves (e.g. Granier and Tardieu 1998) and whole plants (e.g. Atkin *et al.* 2006). However, phenotypic plasticity to spatial temperature differences within a plant has hardly been studied so far, with the exception of temperature differences between the root and the shoot (e.g. Nagel *et al.* 2009). This chapter focuses on the influences of spatial temperature differences between the apical bud and the rest of the plant in *Cucumis sativus* L. (cucumber) plants on leaf expansion and resulting phenotypic adjustments at shoot level.

Leaf initiation rate (LIR) highly depends on temperature (Granier and Tardieu 1998; Granier *et al.* 2002). It is a measure of the rate at which new phytomeres (leaf, internode and axillary bud) are formed by the shoot apical meristem (SAM). In many indeterminately growing dicot species, the SAM is surrounded by the young folded leaves. The SAM and folded leaves form the apical bud, a distinct structure on the top of the shoot (Savvides *et al.* 2013). The apical bud is often subjected to a different microclimate than the lower parts of the shoot due to more exposure of the top of the shoot to the extra-canopy environment (Gibbs and Patten 1970; Leuning and Cremer 1988; Tuzet *et al.* 1997). Therefore, the temperature of the apical bud (T_{bud}) may considerably deviate from the temperature of the rest of the plant (T_{plant}). Detailed research on *Cucumis sativus* revealed that in a range of normal growth temperatures LIR linearly increased with T_{bud}, independent of T_{plant}, even when the difference between T_{bud} and T_{plant} was as large as 8 °C (Chapter 3). This sole temperature dependence of LIR on T_{bud} implies that, at a certain T_{plant}, the shoot will be comprised of more or less phytomeres, depending on whether T_{bud} being higher or lower than T_{plant} respectively. If and how such temperature differences also influence leaf expansion, and its consequences for plant shape and leaf area distribution over the vertical plant axis is unknown.

The leaf area distribution over the shoot is determined by the leaf area per leaf. The final area of a leaf is determined by the mean leaf expansion rate (LER), a

measure of leaf area accumulation per leaf over time, and the duration of leaf expansion (LED), defined as the time period during which a leaf expands. Under uniform plant temperatures, LER and LED have been solely linked to the leaf temperature (T_{leaf}) during leaf expansion: in a range of plant species LER increased and LED decreased with increasing T_{leaf} (e.g. *Helianthus annuus* L., Granier and Tardieu 1998; *Arabidopsis thaliana* L., Granier *et al.* 2002). The increase in LER, with increasing T_{leaf}, may partly or fully counterbalance the decrease in LED resulting in reduced (e.g. *Gossypium barbandense* L., Reddy *et al.* 1993) or not influenced final leaf area (Granier *et al.* 2002) respectively. Under these experimental conditions, LIR well correlated with the subsequent LER and negatively correlated with LED suggesting some kind of coordination with respect to plant temperature (Granier and Tardieu 1998; Granier *et al.* 2002; Parent *et al.* 2010). As a consequence of this assumed coordination, plants might have constant number of leaves expanding (and phytomeres growing) on the shoot at the same time independent of plant temperature. This is supported by a study on *Pisum sativum* L. (pea) in which the number of initiated leaves was proportional to the number of fully expanded leaves when temperature was varied (Turc and Lecoeur 1997).

It is however unknown if and how the leaf expansion might be influenced by alterations in T_{bud}. T_{bud} is actually the temperature of the SAM but also the temperature of the folded leaves surrounding the SAM. A sole increase in T_{bud} increases LIR (Chapter 3) which is probably accompanied by a simultaneous increase in the initial leaf expansion rate of the surrounding folded leaves. Leaf expansion duration is temperature-compensated (Granier and Tardieu 2009). Hence, the increase in T_{bud} is expected to decrease leaf expansion duration. However, this decrease in leaf expansion duration will not be as proportional to LIR as in the case of a uniform increase in plant temperature due to the difference in exposure duration to this increased temperature. This potential shift in the relation between LIR and LED of a certain leaf might cause a change in the number of phytomeres initiated during leaf expansion. Leaf expansion and consequently final leaf area are affected by the number of sink organs (e.g. expanding leaves, fruits) during leaf expansion (Alderfer and Eagles 1976; Marcelis 1993b). For example, when young expanding leaves were removed, leaf expansion rate and final leaf area were higher for the remaining older leaves (*Phaseolus vulgaris*; Alderfer and Eagles 1976). In addition, increasing fruit load significantly reduced

final leaf area (*Cucumis sativus*; Marcelis 1993b). Therefore, altering T_{bud} may influence individual leaf expansion and final leaf area.

The number of leaves and leaf area per leaf are determining plant leaf area and its distribution along the shoot. These are important aspects regarding plant light interception and plant photosynthesis (Falster and Westoby 2003; Sarlikioti *et al.* 2011). Under uniform plant temperatures, plant leaf area and its distribution along the shoot over time are well-defined and predictable which results in a well-defined and predictable light interception. It can be hypothesized that altering T_{bud} compared to T_{plant} would influence plant leaf area and its distribution along the shoot. Increasing T_{bud} would increase the number of phytomeres. However, the faster accumulation of new phytomeres without the coordinated increase leaf area per leaf would result in lower light interception. Therefore, it is unclear what the overall effect of T_{bud} deviating from T_{plant} on plant growth would be. In addition, the altered number of phytomeres without the respective change in light interception and thereby plant photosynthesis, usually observed when plant temperature is uniformly increasing, might affect the sink-source balance of the plant. As organ formation is affected by altered T_{bud} an altered biomass distribution might be expected as well.

Results from a previous study showed that LIR is only dependent of T_{bud} and is not affected by $T_{plant.}$ However, there are indications that when T_{bud} deviates from T_{plant}, a situation that is common in natural plant stands, might affect certain aspects of plant phenotype, like leaf expansion and plant leaf area distribution along the shoot. This might result in alterations in plant growth and biomass allocation. The main goal of this study is to unravel the impacts of spatial temperature differences between the apical bud and the rest of the shoot in *Cucumis sativus* (cucumber) plants on leaf expansion and resulting phenotypic adjustments at shoot level.

Materials and methods

Plant material and growth conditions

Cucumber (*Cucumis sativus* L. cv. Venice RZ) plants were grown in a climate room at 22 ^{o}C air temperature (T_{air}) , 70% relative humidity (RH; VPD = 0.8 kPa) and ~380 μmol mol^{-1} [CO_2] on rockwool slabs and watered with nutrient solution (EC = 2 dS m^{-1}, pH = 5.0 - 5.5). The plants were illuminated by SON-T lamps (MASTER GreenPower CGT 400W E40 1SL; Royal Philips Electronics N.V., Amsterdam, The Netherlands) at a photosynthetic photon flux density (PPFD) of 200 μmol m^{-2} s^{-1} during 16h photoperiod. Two lamps were installed per m^2 to achieve homogeneous distribution of light intensity. The lamps were isolated from the climate cell by a glass ceiling which enabled the separate convective cooling of the lamps and an energy screen (OLS60; *AB Ludvig Svensson*, Kinna, Sweden) was added below the glass ceiling to reduce the thermal radiation emission by the lamps and maintain the homogeneous distribution of light intensity in the climate room. After the 7th leaf had unfolded (~28 days after plant emergence) and the apical buds were distinct structures on the top of the plant canopy (prior this stage apical buds are hidden below the youngest unfolded leaves), plants were subjected to various bud – plant temperature differences.

Temperature treatments

Plants were subjected to 9 different combinations of T_{bud}/T_{plant} in the range of 18-26 ^{o}C (18/18, 22/18, 26/18, 18/22, 22/22, 26/22, 18/26, 22/26, 26/26). The differences between T_{bud} and T_{plant} were achieved by maintaining T_{bud} at 18, 22 or 26 ^{o}C using a custom-made device (Chapter 3) and maintaining T_{plant} (the temperature of the rest of the plant) by controlling T_{air} in three treatments differing in T_{air} (18, 22 and 26 ^{o}C) that were carried out one after the other. Plant temperature, measured at three shoot heights (bud, intermediate leaf, bottom leaf of plants not subjected to bud-plant temperature differences) with thermocouples in a pilot experiment, was equal to T_{air} therefore T_{air} was safely considered as T_{plant} (Chapter 3). Eight plants were subjected to each T_{bud}/T_{plant} combination for 28 days. During plant development side shoots were removed when at maximal 2cm length to allow single-shoot plants. In all treatments, fruits were only allowed to develop at every

4th internode starting from the 10th internode to avoid uneven fruit set and abortion and thereby to keep the photosynthate allocation balanced.

Plant measurements

Number of initiated leaves/phytomeres: The methodology was adopted from Chapter 3. The number of leaves/phytomeres initiated during the treatments was obtained by counting the total number of leaves on plants at the end of the treatments (destructive measurements on 8 plants per treatment). The visible (to the naked eye) leaves were counted by eye while the very young and invisible leaves (leaf primordia) in the apical bud were quantified by dissecting the apical bud under a stereomicroscope (Wild M7 S, Heerbrugg, Switzerland; 60x – 310x). The latest initiated leaf primordium was defined as the latest formed projection that was visible at the side of the meristem (dome).

Leaf expansion analysis: Leaf expansion was defined as the cumulative increase in leaf area. To quantify the relation between leaf expansion and time (t; days) across the treatments, leaf (lamina) length (LL) and (maximum) leaf width (LW) were measured non-destructively every 4 days on 5th, 7th, 9th, 11th, 13th,15th, 17th and 19th leaves starting from the time of leaf unfolding (leaf length + petiole length = 10 cm) until the end of the treatments. To estimate leaf area based on LL x LW, a model relating LL x LW with the actual leaf area was constructed based on destructive measurements of actual leaf area in combination with LL x LW per leaf at the end of the treatments (Fig. S1b). The Pearson correlation coefficients of the relation between the estimated leaf area and the actual leaf area did not significantly differ across treatments (Fig. S1c). The relation between LL and LW was similar across T_{bud}/T_{plant} treatments indicating the absence of leaf shape differences (Fig. S1a).

In order to enable the estimation of leaf expansion rate and duration, the two determinants of leaf area, Gompertz sigmoidal function (Winsor 1932) was fitted to the relation between (estimated) leaf area (A) and time (t).

$$A = A_{max} e^{-e^{a-bt}} \qquad \textbf{(Eqn. 1)}$$

A_{max} is the upper asymptote of the sigmoidal curve or else the estimated maximum leaf area of a fully expanded leaf (eq. 2). A_{max}, a and b the three parameters

characterizing the sigmoidal curve, were estimated using solver add-in in excel based on the minimum root-mean-square error, a measure of the difference between the LL x LW-based leaf area and the Gompertz-based predicted leaf area during leaf expansion.

The (estimated) duration of leaf expansion (LED) was calculated as the period (in days) between leaf initiation and the time at which leaf reached 95% of its A_{max} as calculated from the sigmoidal curve (Granier and Tardieu 1998; Cookson *et al.* 2005). Accordingly, final leaf area (FLA) was defined as the leaf area at the time that leaf reached 95% of its A_{max} as calculated from the sigmoidal curve. The day of leaf initiation was calculated based on the linear relation between the number of leaves initiated and time.

Leaf expansion rate is the leaf area formed per unit of time (m^2 day^{-1}). Leaf expansion rate at time j (LER_j) was calculated from initiation to the end of expansion (or treatment) using the equation (Winsor 1932):

$$LER_j = [bAe^{a-bt}]_j \qquad \textbf{(Eqn. 2)}$$

Mean leaf expansion rate (LER) was calculated as the average leaf expansion rate from leaf initiation to the time that leaf reached 95% of its A_{max} as calculated from the sigmoidal curve (Granier and Tardieu 1998; Cookson *et al.* 2005).

In order to separate between the leaf temperature effects and other potential effects on the leaf expansion components, LER and LED and subsequently on FLA (the 95% of the A_{max}), leaf expansion rate normalized for T_{leaf} – based thermal time (LER_{dd}; m^2 [^oC days]$^{-1}$) and leaf expansion duration in T_{leaf} – based thermal time (LED_{dd}; ^oC days) were calculated. T_{leaf}, the mean leaf temperature during leaf expansion, was based on T_{bud} when the leaf was still a part of the bud (before unfolding) and on T_{air} when the leaf was unfolded away from the bud until leaf reached the 95% of the A_{max}. Thermal time for leaf expansion was calculated using the equation:

$$Thermal\ time = \sum_{n=1}^{k}[(T_{leaf})_n - T_{base}] \qquad \textbf{(Eqn. 3)}$$

T_{base} is the base temperature at which cessation of the developmental process occurs (Trudgill *et al.* 2005). k is the duration of the treatments in days. The T_{base} used was 10 °C (Chapter 3).

FLA and its determinants, LER and LED (and also LER_{dd} and LED_{dd}) , were related to T_{leaf} and T_{bud}-T_{leaf} in a general linear model (see statistical analysis) for the leaves 5, 7 and 9 which have reached their FLA in all treatments before the end of the treatments. T_{bud}-T_{leaf} was the difference between the mean bud temperature and mean leaf temperature during the expansion of each individual leaf (Table S2). T_{bud}-T_{leaf} represents the degree of deviation between T_{bud} and T_{leaf} when compared to uniform plant temperatures.

Plant growth- and architecture-related measurements: Destructive measurements followed the end of the treatments. Plants were removed from the climate room at the end of the night to avoid the build-up of non-structural carbohydrates. The apical buds (SAM and surrounding folded leaves) were excised for the stereoscopic observations (leaf counting) and the rest of the shoot (i.e. all the phytomeres bearing unfolded leaves) was subjected to various measurements. The number of phytomeres bearing unfolded leaves, leaf length, width, area (using LI-3100C Area Meter, Li-Cor Inc., NE, USA), fresh weight (FW), petiole length and FW, internode length, and fruit FW were quantified. The stem was then divided in four segments (1-7, 8-22, 23-30 and >30 phytomeres) and the FW of the segments was also quantified. Shoot (leaves, petioles, stem, fruits) dry weights (DW) per phytomer or segment were measured after drying the shoot components for one day at 80 °C and then for two days at 105 °C. Specific leaf area (SLA; m^2 Kg^{-1}) was estimated as the plant leaf area divided by the total leaf DW. Dry matter content (DMC) was estimated as the percentage of DW in FW. Average leaf area per unfolded leaf was estimated as the plant leaf area divided by the number (of phytomeres bearing) unfolded leaves.

Statistical analysis

The statistical analysis was performed using IBM SPSS statistics v22.0 for Windows (IBM Corp., Armonk, NY, USA). Correlations were tested using the Pearson correlation coefficient (r) and the correlations were considered as significant when $P < 0.01$. One-way analysis of variance (ANOVA) and *post hoc* Tukey's honestly

significant difference (HSD) multiple comparison tests ($P < 0.05$) were used to evaluate statistically significant differences on the mean T_{bud}-T_{plant} across the nine T_{bud}/T_{plant} treatments and T_{bud}-T_{leaf} for the 5th, 7th and 9th leaves across the nine T_{bud}/T_{plant} treatments. A general linear model was fitted to the leaf expansion data using FLA, LER, LED, LER_{dd} and LED_{dd} as response variates. T_{leaf} and T_{bud}-T_{leaf} during leaf expansion were selected as explanatory variates (when $P < 0.05$). A general linear model was also fitted to the data from the destructive measurements using stem length, plant leaf area, SLA, shoot FW, shoot DW and shoot DMC as response variates. T_{plant} and T_{bud}-T_{plant} during the whole plant growth were selected as explanatory variates (when $P < 0.05$).

Results

Realized temperatures

Plants were kept in control conditions for ~28 days (~22 °C air temperature) until the 7th leaf was unfolded. Thereafter, the plants were subjected to different T_{bud}/T_{plant} treatments for 28 days. Set points for T_{plant} were 18, 22 and 26 °C, while realized T_{plant} during the actual treatment period was 17.7, 21.4 and 26.2 °C, respectively (Table S1). During this period T_{bud} deviated from T_{plant}, ranging from -7.3 (18/26) to 8.3 °C (26/18; Table S1). The different phenotypic traits that were measured at the end of the experiment (Table 2) were analyzed in relation to the (mean) T_{plant} and T_{bud}-T_{plant} over the whole plant growth period (i.e. from plant emergence to the end of the treatments). During the whole plant growth period the average realized T_{plant} was 19.8, 21.8 and 24.3 °C for the three treatments with T_{plant} set points of 18, 22 and 26 °C, respectively (Table S1). Over the whole growth period T_{bud} deviated from T_{plant} from -3.5 (18/26) to 4.2 °C (26/18; Table S1).

The leaf expansion characteristics LED, LER and FLA, obtained from measurements on the 5th, 7th and 9th leaf were analyzed in relation to their own temperature (T_{leaf}) and the realized difference between bud and leaf temperature (T_{bud}-T_{leaf}) during their expansion (Table 1 and S2). All three leaves included in the leaf expansion analysis were already initiated before the start of the treatments and expanded during the treatments (Fig. 1). Therefore, both T_{leaf} and T_{bud}-T_{leaf} were

functions of both the pre-treatment and treatment temperatures depending on the timing of leaf initiation and the leaf expansion duration of each leaf.

From leaf initiation to leaf expansion

The total number of initiated phytomeres on the plants at the end of the treatments linearly increased with increasing T_{bud} regardless T_{plant} (Fig. 1a). The increase in the number of phytomeres bearing unfolded leaves was proportional to the increase in the number of initiated phytomeres across the different T_{bud}/T_{plant} treatments (Fig. 1b). The proportional increase in the number of initiated and unfolded leaves with increasing T_{bud} indicates that the leaf expansion rate from leaf initiation to leaf unfolding was a function of T_{bud}, which determines T_{leaf} from leaf initiation to unfolding.

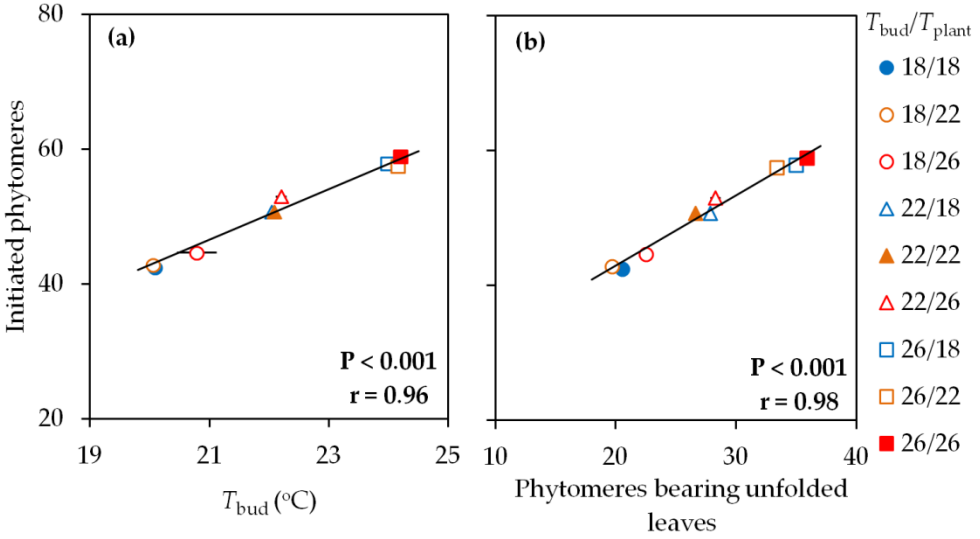

Fig. 1. The total number of initiated phytomeres per plant in relation to bud temperature (T_{bud}; a) and the total number of initiated phytomeres in relation to the total number of phytomeres bearing unfolded leaves at the end of the treatments (b; n=8). Where statistically significant correlations were found ($P < 0.01$) the Pearson correlation coefficient (r) was indicated on the graphs.

When plant temperature increased from 18 to 26 °C with bud temperature being equal to plant temperature, expanding unfolded leaves showed increased

leaf area accumulation (expansion) while their expansion duration was shorter (Fig. 2a, e and i). The number of fully expanded leaves on the shoot increased with increasing plant temperature (Fig. 2a, e and i). In accordance to the leaf expansion curves (Fig. 2), LER increased and LED decreased with increasing T_{leaf} for each individual leaf (Table 1).

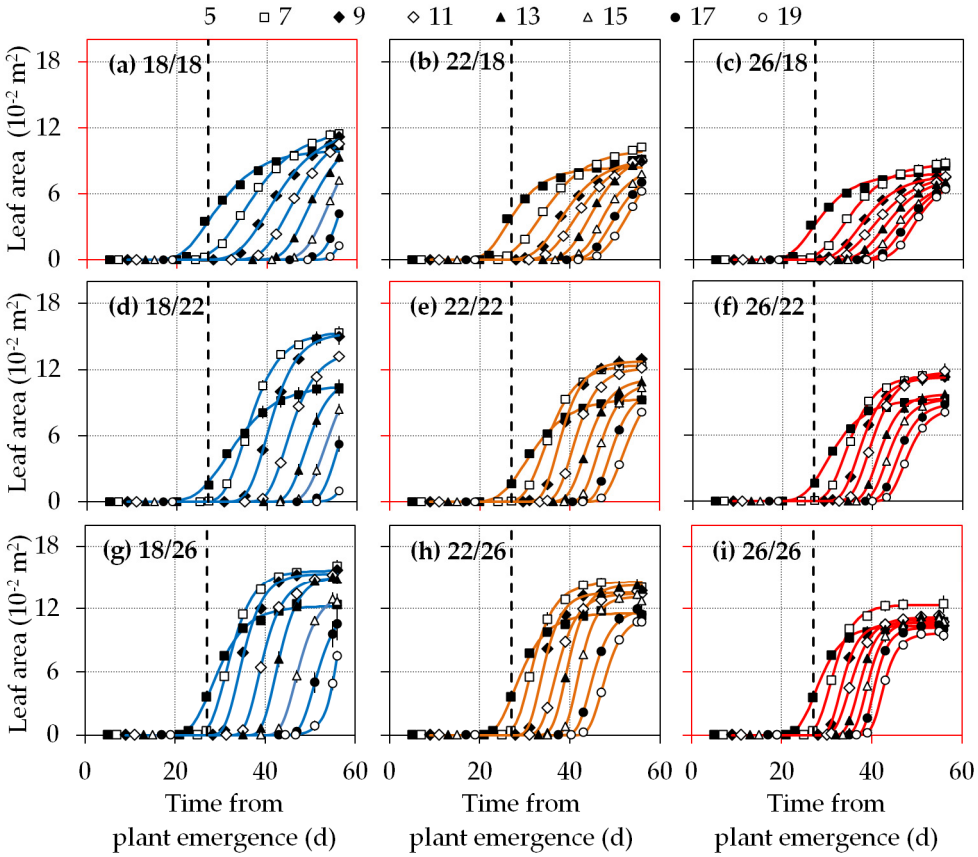

Fig. 2. Leaf expansion (the cumulative increase in leaf area, estimated from leaf length and width) from plant emergence until the end of the treatments of 5th, 7th, 9th, 11th, 13th, 15th, 17th and 19th leaves under the nine different T_{bud}/T_{plant} treatments (n=8). Data points were fitted by the Gompertz curve (n=8). The vertical dashed line represents the start of the treatments. Graphs with red outline are the control treatments (i.e. 18/18, 22/22, 26/26).

Table 1. Estimated mean leaf expansion rate (LER; 10^{-2} m^2 day^{-1}), leaf expansion duration (LED; days) and final leaf area (FLA; 10^{-2} m^2) of the 5th, 7th and 9th leaves (n=8) in relation to the mean leaf temperature (T_{leaf}; Table S2) and the deviation between the mean bud and mean leaf temperature (T_{bud}-T_{leaf}; Table S2) during the leaf expansion under the nine T_{bud}/T_{plant} treatments. The regression coefficient (b) for each factor (T_{leaf} or T_{bud}-T_{leaf}) represents the (positive or negative (-)) change in the dependent variable (i.e. LER, LED or FLA) per 1 °C increase in the factor under the assumption that the relation between the dependent variable and the factor is linear. Statistically significant effects with $P < 0.001$(***), $P < 0.01$(**), $P < 0.05$ (*) and non-statistically significant with $P > 0.05$ (n.s.).

Leaf #	Leaf trait	T_{bud}/T_{plant} treatment									General linear model		
											Regression coefficients (b)		Adj. R^2
		18/18	22/18	26/18	18/22	22/22	26/22	18/26	22/26	26/26	T_{leaf}	T_{bud}-T_{leaf}	
5th	LER	0.23	0.22	0.20	0.22	0.21	0.22	0.32	0.31	0.30	*** 0.02	n.s.	0.51
	LED	40.1	35.0	36.3	44.5	41.5	39.3	36.0	34.7	32.4	***-2.18	***-1.36	0.25
	FLA	9.5	8.0	7.4	10.1	8.9	8.8	11.6	11.0	9.8	n.s.	***-0.65	0.49
7th	LER	0.22	0.21	0.19	0.35	0.30	0.28	0.43	0.41	0.36	*** 0.03	***-0.02	0.90
	LED	46.9	44.6	43.2	41.0	39.3	38.1	34.4	33.5	32.1	***-3.51	***-0.77	0.84
	FLA	10.6	9.6	8.4	14.7	11.8	10.9	14.9	13.9	11.7	n.s.	***-0.94	0.75
9th	LER	0.21	0.19	0.17	0.34	0.32	0.28	0.42	0.39	0.32	*** 0.02	***-0.03	0.85
	LED	47.9	45.2	42.3	42.1	37.6	37.7	34.2	32.2	30.9	***-3.62	***-0.45	0.89
	FLA	10.2	8.8	7.1	14.6	12.1	10.7	14.6	12.9	10.1	n.s.	***-1.08	0.66

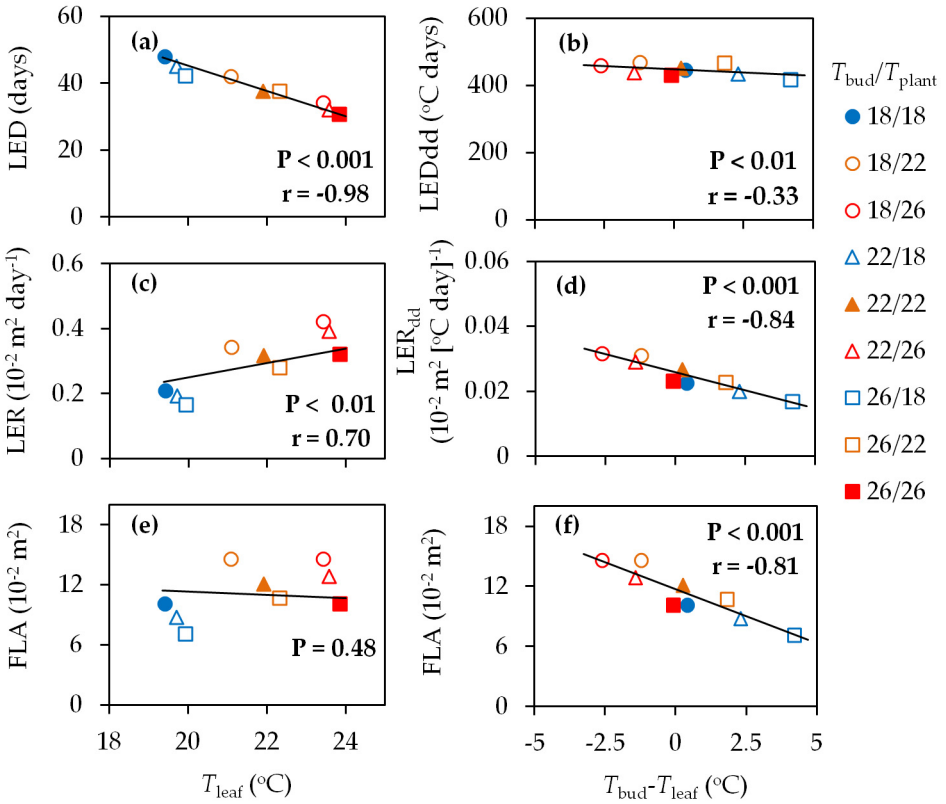

Fig. 3. On the left, leaf expansion duration (LED; a), mean leaf expansion rate (LER; c), and final leaf area (FLA; e) of the 9th leaf in relation to the mean leaf temperature during leaf expansion (T_{leaf}) across the nine different T_{bud}/T_{plant} treatments (n=8). Correlations (lines) were based only on the data points from the treatments in which bud temperature (T_{bud}) was not altered from the temperature of the rest of the plant (closed symbols; treatments 18/18, 22/22, 26/26). On the right, leaf expansion duration in T_{leaf}-based thermal time (LED_{dd}; b), mean leaf expansion rate normalised for T_{leaf}-based thermal time (LER_{dd}; d), and FLA (f) of the 9th leaf in relation to the difference between the mean bud temperature and mean leaf temperature (T_{bud}-T_{leaf}) during the expansion of the 9th leaf across the nine different T_{bud}/T_{plant} treatments. Correlations (lines) were based on the data points from all the nine treatments. Where statistically significant correlations were found ($P < 0.01$) the Pearson correlation coefficient (r) have been indicated on the graphs.

Final leaf area (FLA) was not influenced by T_{leaf} (Table 1) indicating that the increase in LER was fully counterbalanced by the decrease in LED with increasing

T_{leaf}. However, altering T_{bud} below or beyond T_{plant} resulted in significant changes in FLA (Table 1).

Despite being influenced by T_{leaf}, LER and LED significantly correlated with T_{bud}-T_{leaf} (Table 1). LER and LED both decreased with increasing T_{bud}-T_{leaf} (Table 1). The negative effect of T_{bud}-T_{leaf} on LED became smaller with increasing leaf number from 5th to 9th while the effect on LER was significant for the 7th and 9th leaves (Table 1).

The decrease in LER and/or LED with increasing T_{bud}-T_{leaf} resulted in decreased FLA regardless T_{leaf} (Table 1). As explicitly shown for the 9th leaf, LED decreased (Fig. 3a) and LER increased (Fig. 3c) with increasing T_{leaf} across the nine different T_{bud}/T_{plant} treatments. However, in the treatments where T_{bud}-T_{leaf} was not equal to zero, LER and, in a lesser degree, LED decreased with increasing T_{bud} at a certain T_{plant} (Fig. 3a and c): under the same T_{plant}, negative T_{bud}-T_{leaf} (e.g. in 18/26 treatment) resulted in higher LED and LER and positive T_{bud}-T_{leaf} (e.g. in 26/18 treatment) resulted in lower LED and LER in comparison to the absence of T_{bud}-T_{leaf} (e.g. in 26/26 and 18/18 treatments respectively; Fig. 3a and c). These deviations in LER and LED were reflected in the relation between FLA and T_{leaf} (Fig. 3e): at the same T_{plant}, negative T_{bud}-T_{leaf} resulted in higher FLA and positive T_{bud}-T_{leaf} resulted in lower FLA in comparison to the absence of T_{bud}-T_{leaf} (Fig. 3e).

Expressing the mean leaf expansion rate and leaf expansion duration in thermal time (LER_{dd} and LED_{dd} respectively) allowed the normalization of the effects of T_{leaf} on the two determinants of FLA (Table S3). In the case of the 9th leaf, LER_{dd} (Fig. 3b) and, in a lesser degree, LED_{dd} (Fig. 3d) decreased linearly with increasing T_{bud}-T_{leaf}. Consequently, FLA was linearly reduced with increasing T_{bud}-T_{leaf} (Fig. 3f).

From leaf area per leaf to the spatial distribution of plant leaf area

When plant temperature increased from 18 to 26 ^{o}C while bud and plant temperature were equal, leaves were initiated faster (Fig. 1a) and expanded faster (Table 1) with shorter expansion duration (Table 1) while their FLA remained unchanged (Table 1). This resulted in a linear increase in plant leaf area accumulation (Table 2). However, the average area per unfolded leaf remained constant with increasing plant temperature in the absence of T_{bud}-T_{plant} (Fig. 4a).

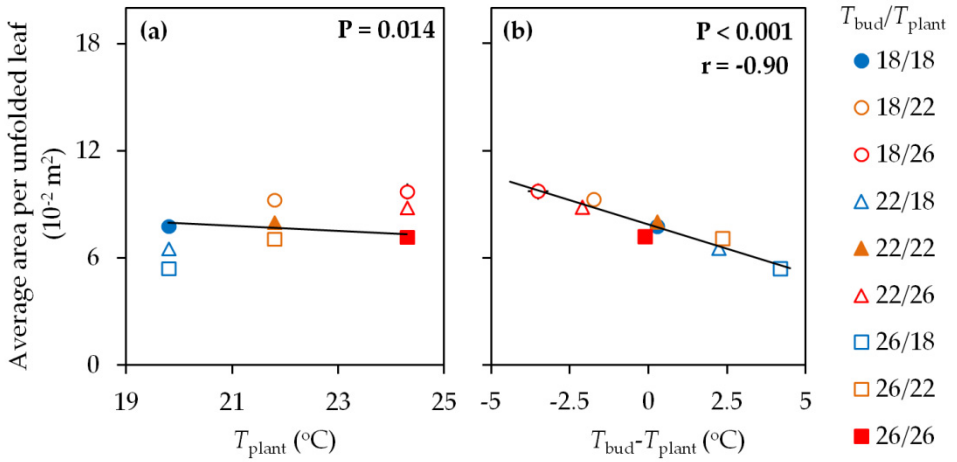

Fig. 4. Average area per unfolded leaf (plant leaf area divided by the number of unfolded leaves at the end of the treatments) in relation to the plant temperature (T_{plant}; a) or bud-plant temperature difference (T_{bud}-T_{plant}; b) across the nine different T_{bud}/T_{plant} treatments (n=8). Correlations (lines) were based only on the data points from the treatments in which bud temperature was not altered from the temperature of the rest of the plant (a; closed symbols; treatments 18/18, 22/22, 26/26) or based on the data points from all the nine treatments (b). Where statistically significant correlations were found ($P < 0.01$) the Pearson correlation coefficient (r) has been indicated on the graphs.

At a given T_{plant}, plant leaf area increased with increasing T_{bud}-T_{plant} (Table 2). However, when T_{bud} differed from T_{plant}, the average area per unfolded leaf was significantly altered: At a given T_{plant} (e.g. 22 °C), negative T_{bud}-T_{plant} (e.g. 18/22) resulted in higher average area per unfolded leaf. Positive T_{bud}-T_{plant} (e.g. 26/22) resulted in lower average area per unfolded leaf in comparison to the absence of T_{bud}-T_{plant} (e.g. 22/22; Fig. 4a). Average area per unfolded leaf was linearly related to the T_{bud}-T_{plant} (Fig. 4b). The linear relation of the average area per unfolded leaf to T_{bud}-T_{plant} indicates that increasing T_{bud} beyond T_{plant} resulted in increasing number of leaves but decreasing leaf area per leaf whereas decreasing T_{bud} below T_{plant} resulted in decreasing number of leaves but increasing leaf area per leaf. This is in accordance with the lower LER of expanding leaves (Fig. 3d) and the lower FLA of expanded leaves with increasing T_{bud}-T_{leaf} (Fig. 3f).

Plant leaf area distribution along the shoot was illustrated by the relation between the phytomer number on the shoot and the leaf area per phytomer (Fig. 5a

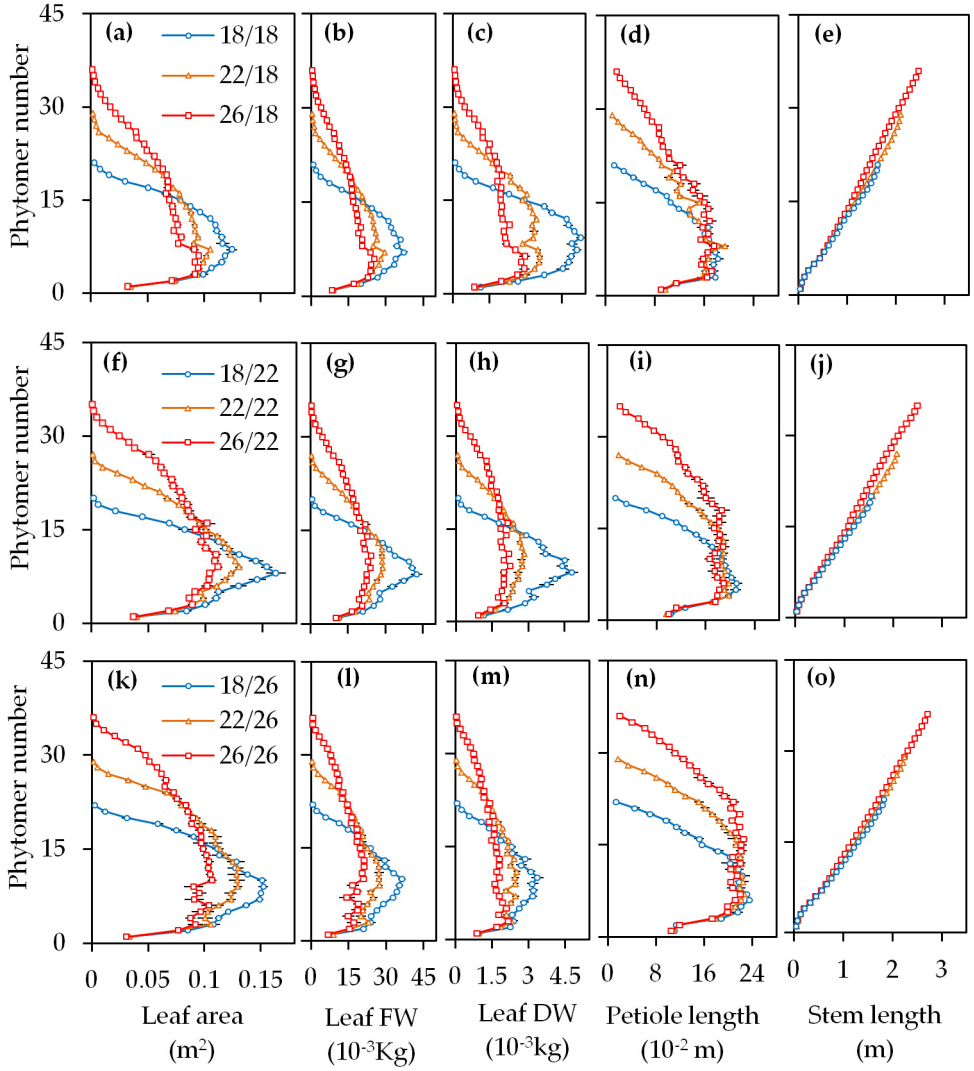

Fig. 5. The distribution of leaf area, leaf fresh weight (FW), leaf dry weight (DW), petiole length per phytomer and stem length of plants treated with different T_{bud} under 18 (top), 22 (middle) and 26 °C (bottom) T_{plant} (n=8).

, f and k). Plant leaf area profile along the shoot followed a common pattern across the T_{bud}/T_{plant} treatments (Fig. 5a, f and k). Leaf area per leaf increased with phytomer number for the basal leaves, the intermediate leaves showed the maximal area while leaf area per leaf decreased thereafter with phytomer number

along the shoot for the young upper leaves. However, as indicated briefly by the average area per unfolded leaf, at a given T_{plant}, plants showed more phytomeres with lower leaf area per phytomer with increasing T_{bud}.

Beside area per leaf and the number of phytomeres, other plant traits determine the spatial distribution of plant leaf area such as the stem (or internode) length and the petiole length. Stem length mainly followed T_{bud} and therefore the number of phytomeres (Table 2; Fig. 5e, j and o). The pattern of petiole length per phytomer was similar to the pattern of the leaf area per leaf along the shoot. However, the length of fully elongated petioles did not show large differences across bud-plant temperature differences (Fig. 5d, i and n).

Plant growth and biomass distribution

When plant temperature increased from 18 to 26 ^{o}C while bud and plant temperature were equal, shoot fresh weight (FW) and dry weight (DW) significantly increased (Table 2). The increase in shoot DW was relatively smaller than the increase in FW due to a decrease in shoot dry matter content (DMC) with increasing temperature (Table 2). The increase in shoot FW was mainly related to the increase in fruit FW and less related to the increase in petiole and stem FW while leaf FW was not significantly influenced. The increase in shoot DW was mainly related to the increase in fruit DW and less related to the increase in stem DW while petiole DW was not significantly influenced. The increase in fruit and stem DW was partly counterbalanced by the decrease in leaf DW with increasing temperature (Table 2). DMC decreased in leaves, fruits, petioles and stem with increasing temperature. The increase in plant leaf area and the decrease in leaf DW resulted in increased specific leaf area (SLA; Table 2).

Shoot FW increased statistically significantly with increasing T_{bud}-T_{plant} while shoot DW was not influenced (Table 2). The unaffected DW resulted from a significant reduction in shoot DMC with increasing T_{bud}-T_{plant}. The small increase in shoot FW (in relation to effects of uniform plant temperature increase) was mainly related to the increase in petiole, stem and fruit FW while leaf FW was not significantly influenced. The stability in shoot DW was related to the increase in fruit and stem DW which was counterbalanced by a significant decrease in leaf DW. Petiole DW was not significantly influenced. DMC decreased in leaves, stem, petioles and fruits with increasing T_{bud}-T_{plant}.

Table 2. Stem length (m), plant leaf area (m^2), specific leaf area (SLA; m^2 $Kg^{-1}DW$) shoot fresh weight (FW; Kg), shoot dry weight (DW; 10^{-2} Kg) and shoot dry matter content (DMC; %) in relation to plant temperature (T_{plant}; Table S1) and the deviation between the bud and plant temperature from plant emergence to the end of the treatments (T_{bud}-T_{plant}; Table S1) under the nine T_{bud}/T_{plant} treatments (n=8). The regression coefficient (b) for each factor (T_{plant} or T_{bud}-T_{plant}) represents the (positive or negative [-]) change in the dependent variable (e.g. SLA) per 1 °C increase in the factor under the assumption that the relation between the dependent variable and the factor is linear. Statistically significant effects with $P < 0.001$(***), $P < 0.01$(**), $P < 0.05$ (*) and non-statistically significant with $P > 0.05$ (n.s.).

Plant trait	T_{bud}/T_{plant} treatments									General linear model		
	18/18	22/18	26/18	18/22	22/22	26/22	18/26	22/26	26/26	Regression coefficients (b)		Adj. R^2
										T_{plant}	T_{bud}-T_{plant}	
Stem length	1.62	2.10	2.46	1.56	2.08	2.50	1.82	2.24	2.71	*** 0.25	*** 0.22	0.89
Plant leaf area	1.61	1.83	1.89	1.82	2.13	2.36	2.18	2.49	2.58	*** 0.23	*** 0.10	0.78
SLA	24.5	28.6	34.1	34.8	43.8	49.1	47.0	52.7	56.6	*** 7.70	*** 2.83	0.87
Shoot FW	0.76	0.85	0.86	0.96	1.25	1.41	2.79	3.12	3.28	*** 0.58	** 0.08	0.89
Leaves	0.45	0.48	0.47	0.47	0.49	0.51	0.49	0.49	0.48	n.s.	n.s.	-
Petioles	0.15	0.17	0.18	0.18	0.21	0.22	0.24	0.28	0.30	*** 0.03	*** 0.01	0.80
Stem	0.13	0.15	0.16	0.15	0.17	0.19	0.16	0.17	0.18	*** 0.01	*** 0.01	0.69
Fruits	0.02	0.05	0.05	0.15	0.38	0.49	1.91	2.17	2.32	*** 0.53	* 0.06	0.87
Shoot DW	8.67	8.78	8.03	7.97	8.46	8.83	11.83	12.36	12.38	*** 0.84	n.s.	0.69
Leaves	6.59	6.41	5.57	5.25	4.88	4.84	4.66	4.73	4.57	***-0.47	***-0.15	0.70
Petioles	0.91	0.97	0.95	0.94	0.94	0.93	0.92	1.00	0.99	n.s.	n.s.	-
Stem	1.04	1.15	1.22	1.11	1.21	1.25	1.12	1.17	1.20	*** 0.04	*** 0.03	0.46
Fruits	0.13	0.25	0.28	0.67	1.43	1.81	5.12	5.47	5.63	*** 1.31	** 0.16	0.91
Shoot DMC	11.4	10.4	9.4	8.4	6.8	6.3	4.3	4.0	3.8	***-1.78	***-0.40	0.95
Leaves	14.5	13.4	12.0	11.2	10.0	9.6	9.6	9.6	9.5	***-1.15	***-0.38	0.67
Petioles	6.0	5.7	5.3	5.2	4.5	4.2	3.8	3.6	3.4	***-0.63	***-0.18	0.93
Stem	8.0	7.7	7.6	7.3	6.9	6.7	7.0	6.7	6.6	***-0.33	***-0.11	0.71
Fruits	5.8	5.5	5.7	4.5	3.9	3.7	2.7	2.5	2.4	***-0.78	**-0.10	0.92

The increase in plant leaf area and the decrease in leaf DW with increasing T_{bud}-T_{plant} resulted in increased SLA (Table 2).

Shoot FW was slightly influenced and shoot DW was not influenced by T_{bud}-T_{plant}. However, the distribution of both FW and DW along the shoot and per phytomer was greatly altered (Fig. 6) similarly to the distribution of plant leaf area. In the case of leaves, increase in T_{bud} beyond T_{plant} resulted in biomass investment towards more leaves accompanied with a decrease in biomass allocation per leaf

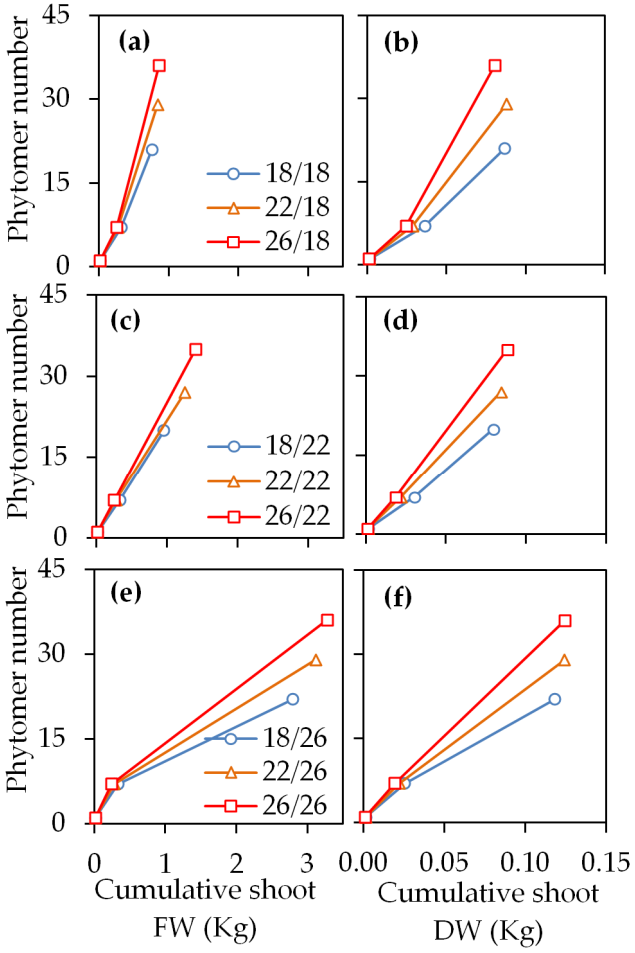

Fig. 6. Phytomer number in relation to the cumulative shoot fresh- (FW; left) and dry-weight (DW; right) of plants subjected to the nine T_{bud}/T_{plant} treatments (n=8).

(especially obvious in basal and intermediate leaves) when compared to the absence of T_{bud}-T_{plant} (Fig. 5b-c and g-h).

At a given T_{plant}, decrease in T_{bud} below T_{plant} resulted in biomass investment towards less leaves accompanied with an increase in biomass allocation per leaf (especially observed in basal and intermediate leaves) when compared to the absence of T_{bud}-T_{plant} (Fig. 5g-l and h-m). The differences observed in biomass allocation per leaf decreased with increasing T_{plant}. Similar FW and DW distributions along the shoot and per phytomer were observed for the petioles, stem and fruits (data not shown).

Discussion

Altered apical bud temperature, in *Cucumis sativus* plants, resulted in strong effects on plant phenotype and no substantial effects on plant growth. The effects on plant phenotype were primarily an outcome of the sole dependence of LIR to T_{bud} (Chapter 3).

More leaves – smaller leaves and vice-versa

The number of phytomeres (bearing unfolded leaves) on the shoot (Fig. 1) and the shoot height (Table 2) solely and mainly followed T_{bud} respectively.

When T_{bud} and T_{plant} were equal, plant leaf area substantially increased with increasing plant temperature (Table 2) while average leaf area per unfolded leaf remained constant (Fig. 4a). In addition, LIR and LER were linearly related to T_{bud} (Chapter 3) and T_{leaf} during leaf expansion (Fig. 3c) respectively while LED was negatively linearly related to T_{leaf} (Fig. 3a) in agreement with previous studies (Granier and Tardieu 1998; Granier *et al.* 2002). This indicates a correlation between leaf initiation, the subsequent leaf expansion and the production of fully expanded leaves (Turc and Lecoeur 1997; Granier *et al.* 2002). It is also suggesting that the number of leaves expanding on the shoot is maintained with increasing plant temperature in *Cucumis sativus* plants in agreement with a study on *Pisum sativum* (Turc and Lecoeur 1997). In this study, the increase in LER fully counterbalanced the decrease in LED resulting in no changes in the FLA with increasing T_{leaf} (Table

1 and Fig. 3e). Overall, with uniformly increasing plant temperature, plant leaf area was more rapidly accumulating on the shoot without changes in leaf area per leaf.

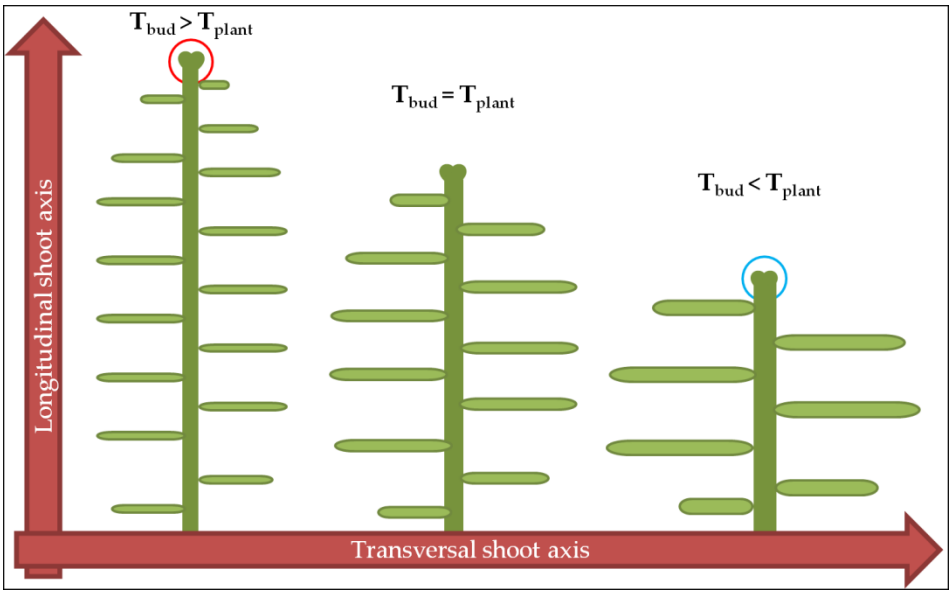

Fig. 7. Two-dimensional illustration of the phenotypes of cucumber plants when subjected to altered apical bud temperature. At a given T_{plant}, increasing T_{bud} above T_{plant} resulted in taller plants with more phytomeres, however, less biomass and leaf area were distributed per phytomer (left) in comparison to plants in the absence of bud-plant temperature difference (middle). At a given T_{plant}, decreasing T_{bud} below T_{plant} resulted in shorter plants with less phytomeres but more biomass and leaf area were distributed per phytomer (right) in comparison to plants in the absence of bud-plant temperature difference (middle).

On the contrary, the alteration of T_{bud} from T_{plant} resulted in substantial changes in leaf area per leaf (Fig. 5a, f and k). A sole increase or decrease in T_{bud} resulted in increased or decreased plant leaf area respectively (Table 2). However, the effect of the sole change in T_{bud} was much smaller than a comparable change in both T_{bud} and T_{plant} (Table 2). The increase in plant leaf area with increasing T_{bud} at constant T_{plant} was solely due to the increase in the number of unfolded leaves and vice versa. Average leaf area per unfolded leaf was negatively linearly related to T_{bud}-T_{plant} (Fig. 4b). This indicates that with increasing T_{bud} beyond T_{plant} the increase in the number of unfolded leaves was partly compensated by a decrease in leaf area

per leaf. On the other hand, with decreasing T_{bud} below T_{plant} the decrease in the number of unfolded leaves was partly compensated by an increase in leaf area per leaf. Consequently, increasing T_{bud} beyond T_{plant} resulted in more and smaller leaves, while decreasing T_{bud} below T_{plant} resulted in less and larger leaves (Fig. 7).

The changes caused by altering T_{bud} on leaf area per leaf were related to changes in LED and/or LER depending on the leaf number (Table 1). LED_{dd} and/or LER_{dd} linearly decreased with increasing T_{bud}-T_{leaf} during leaf expansion resulting in reduced FLA (Table 1). Previous studies suggested that leaf expansion can be solely related to T_{leaf} (Granier and Tardieu 1998; Granier *et al.* 2002). In this study we have shown that this relation stands only when plants are subjected to uniform plant temperatures (Fig. 3a). The correlations between the leaf expansion determinants (normalized for T_{leaf} effects) and FLA with T_{bud}-T_{leaf} irrespective of T_{leaf} imply that leaf expansion is not a function of T_{leaf} alone but at least a function of two different temperatures across the plant, namely T_{bud} and T_{leaf}, during leaf expansion.

T_{bud} during leaf expansion is influencing the number of phytomeres initiated and therefore the number of growing organs present on the shoot during the expansion of a certain leaf while T_{leaf} is regulating the duration of leaf expansion. The more the T_{bud} increases or decreases in relation to T_{leaf} the more the number of growing organs will increase or decrease respectively during the expansion of a certain leaf. The number of growing organs during leaf expansion was shown to affect leaf expansion and FLA (Alderfer and Eagles 1976; Marcelis 1993b). In this study, the accumulation of more or less phytomeres over time without the respective increase or decrease in the whole plant temperature was accompanied with smaller or larger leaves respectively. This suggests that the effect of altered T_{bud} on leaf expansion and FLA is related to the number of growing phytomeres during leaf expansion. This is in agreement with the similar FLA observed with uniformly increasing plant temperature as the number of growing phytomeres during leaf expansion was maintained as suggested by the negative proportional relation between LIR (Chapter 3) and LED (Fig. 3a) with increasing plant temperature.

Previous studies suggested that leaf initiation and subsequent leaf expansion are coordinated processes across temperatures even under temporal temperature fluctuations (Granier and Tardieu 1998; Granier *et al.* 2002; Parent *et al.*

2010). This coordination was based on similar correlations of LIR, LER and 1/LED with temperature (Granier *et al.* 2002). In this study, we have interfered in these correlations obtained under uniform plant temperatures by altering T_{bud} and subsequently LIR while maintaining T_{plant}. Our findings suggest that the correlation observed between leaf initiation and leaf expansion with increasing plant temperature is based on the constant number of growing phytomeres (or leaves) on the plant. Increasing or decreasing the number of growing phytomeres due to a sole increase or decrease in T_{bud} decreases or increases individual leaf expansion respectively. These findings strengthen the notion on strict coordination between different developmental processes, like leaf initiation and leaf expansion. In agreement with Granier and Tardieu (2009), our findings show that leaf expansion is determined by mechanisms at different organizational levels.

More phytomeres – less biomass per phytomer and vice versa

When T_{bud} and T_{plant} were equal, plant growth was substantially enhanced with increasing plant temperature as indicated by the final increase in shoot FW and DW (Table 2) in the range of 18-26 ^{o}C. This is in agreement to previous studies (e.g. Grimstad and Frimanslund 1993). In addition, the increase in plant temperature shifted biomass distribution from vegetative (i.e. leaves) to generative organs (i.e. fruits; Table 2) and increased SLA for cucumber plants as suggested by Marcelis (1993a; 1993b) indicating thinner leaves.

Plant growth was not substantially influenced by the sole alteration in T_{bud} in comparison with changes in both T_{bud} and T_{plant} (Table 2). Therefore, depending on whether T_{bud} increased beyond or decreased below T_{plant} similar amount of resources were allocated into more or less phytomeres respectively (Fig. 6). The greater the increase of T_{bud} beyond or the decrease below T_{plant}, the lower or the higher the biomass per phytomer was respectively. In addition, the decrease in biomass per phytomer with increasing in T_{bud}-T_{plant} (Fig. 6) was accompanied with a shift in biomass allocation from leaves to fruits and increased SLA (Table 2) indicating thinner leaves.

The increase of T_{bud} beyond T_{plant} maintained the rate of plant growth but increased the number of growing phytomeres (sinks). The decrease of T_{bud} below T_{plant} maintained the rate of plant growth but decreased the number of growing

phytomeres. Consequently, altered T_{bud} did not affect plant source strength (photosynthate supply) but substantially affected the number of sinks.

Phenotypic plasticity to altered apical bud temperature

Within a normal growth temperature range, cucumber plants subjected to altered T_{bud} at a given T_{plant} showed important phenotypic adjustments from leaf to plant level while maintaining their growth potential. According to De Kroon (2005), phenotypic plasticity in plants is expressed at a subindividual level (i.e. organ or module). This implies that individual organs, such as meristems and leaves, respond to changes and differences in local environmental conditions (De Kroon *et al.* 2005). The sole dependence of LIR to T_{bud} facilitates the local perception of the extra-canopy environment and the local decision on the rate at which the plant will continue growing towards this environment (Chapter 3). In case of the local perception of a more optimum extra-canopy environment, resulting in more optimum plant temperature (which is most of the times correlated with higher levels of shortwave radiation and hence higher light levels), higher LIR facilitates the faster development of a plant towards the more optimum-for-growth environment (Fig. 7). In case of the perception of a less optimum extra-canopy environment lower LIR facilitates the slower development of a plant towards the less optimum-for-growth environment (Fig. 7).

However, the sole increase or decrease in the number of phytomeres without the respective arrangements at plant level cannot yield a successful plant strategy to cope with these intra-plant temperature differences. Whole-plant plasticity is the sum of all organs responses triggered by local environmental conditions in combination with all the interactions effects that are due to communication and behavioural integration of these organs (De Kroon *et al.* 2005). When T_{bud} increased beyond T_{plant}, the faster accumulation of growing phytomeres/leaves was followed by a decreased leaf area and biomass per phytomer (Fig. 7). On the other hand, when T_{bud} decreased below T_{plant}, the slower accumulation of growing phytomeres/leaves was followed by an increased leaf area and biomass per phytomer (Fig. 7). From a certain point of view, this shows the importance of apical bud and LIR in determining the leaf area and biomass distribution along the shoot. From another point of view, shifting investments between different plant parts increases plant fitness (Sadras and Denison 2009).

Plants subjected to increased T_{bud} (relative to T_{plant}) facilitated an extended growth towards the longitudinal shoot axis by limiting their investments per phytomer, while plants subjected to decreased T_{bud} facilitated an extended growth towards the transversal shoot axis by enhancing their investments per phytomer (Fig. 7). Therefore in both cases, plants have invested more towards the most optimum environment for growth.

Implications in the study of plant ecophysiology and future considerations

Spatial plant temperature heterogeneities are common and they are definitely not the exception. They were quantified in nature (Gibbs and Patten 1970), field crop cultivation (Gardner *et al.* 1981) and in protected cultivation (Kempkes and van de Braak 2000; Li *et al.* 2014). We have here shown that altered apical bud temperature critically alters plant phenotype. The physiological mechanisms, however, behind these phenotypic alterations, like the response of leaf expansion remain to be unraveled.

Common experimental practices assume uniform plant temperatures that can be safely approximated either by quantifying air temperature or the temperature of, for example, a single leaf within the plant canopy. Taking into consideration 1) that plant temperature can greatly differ from air temperature even under moderate environments (e.g. Savvides *et al.* 2013), 2) that plant temperature can be spatially heterogeneous (e.g. Kempkes and van de Braak 2000) and 3) that spatial temperature heterogeneities can cause tremendous alterations in plant phenotype, plant temperature should be properly spatiotemporally quantified or estimated to avoid the misinterpretation of experimental results.

Coupling fundamental processes, like leaf initiation and leaf expansion, with the temperatures actually perceived by the apical bud or the leaf respectively in plant growth models is necessary (Chapter 3) but not enough. This study shows that plants are not just the sum of modules that are independently contributing to plant growth based on the local environmental perception (e.g. T_{leaf}) but a sum of interconnected and highly interacting modules able to shape plant phenotype to satisfy plant needs even under spatial plant temperature heterogeneities. This knowledge can be integrated in plant growth modeling by linking organ microclimate models (Chelle 2005) with functional structural plant models (Vos *et al.* 2010) using a systems biology approach (Baldazzi *et al.* 2012; Poorter *et al.* 2013).

Conclusions

Shoot spatial temperature differences, even though common in nature and cultivated crops, did not attract much attention in the past years. We here investigated the effects of altered apical bud temperature on individual leaf expansion and whole plant phenotype in cucumber plants. Our findings provide new insights to the current knowledge on the coordination between leaf initiation and leaf expansion and unravel substantial phenotypic changes to altered bud temperature at plant level. Increasing T_{bud} beyond T_{plant} resulted in more and smaller leaves while decreasing T_{bud} below T_{plant} resulted in less and larger leaves. This offset between leaf number and individual leaf area indicates a strict systemic coordination between leaf initiation and leaf expansion. The same patterns as leaf area distribution were observed for biomass distribution across phytomeres. Cucumber plants adjust their phenotype to increased or decreased T_{bud} by reallocating their investments into more or less phytomeres respectively. The phenotypic plasticity to altered apical bud temperature in cucumber plants shows that plants are able to adapt to temperature heterogeneities by 1) responding to the local temperature at organ level with 2) simultaneous adjustments at plant level that will secure plant survival and growth. Intra-plant temperature differences should be seriously taken into consideration during experimentation but also introduced in plant growth models.

Acknowledgments

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Appendix

Fig. S1. Relation between leaf area and [leaf length x leaf width] (a) the relation between leaf length and leaf width (b) and the relation between the actual leaf area and the estimated leaf area based on the model (c) in cucumber plants subjected to the nine different T_{bud}/T_{plant} treatments.

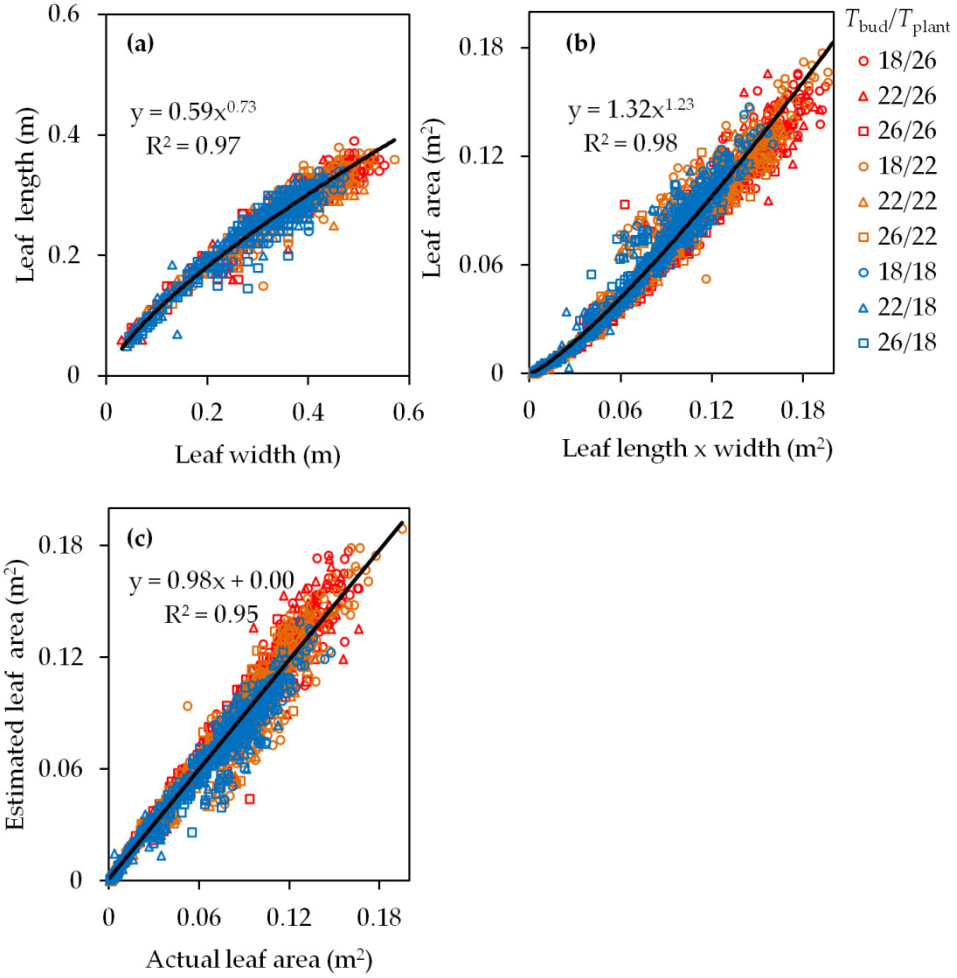

Table S1. Major plant temperature (T_{plant}) and the deviation between bud and major plant temperature (T_{bud}-T_{plant}) during the treatments (n=8) and from plant emergence until the end of the treatments (during plant growth) under the nine T_{bud}/T_{plant} treatments. Different letters within a row represent statistically significant differences ($P < 0.05$).

Temperatures		T_{bud}/T_{plant} treatment								
		18/18	22/18	26/18	18/22	22/22	26/22	18/26	22/26	26/26
During treatment	T_{plant}		17.7			21.4			26.2	
	T_{bud} -T_{plant}	-0.5c	4.5b	8.3a	-3.4d	-0.7c	4.7b	-7.3e	-4.1d	-0.1c
During growth	T_{plant}		19.8			21.8			24.3	
	T_{bud} -T_{plant}	0.3c	2.2b	4.2a	-1.7d	0.3c	2.4b	-3.5e	-2.1d	-0.1c

Table S2. Leaf temperature (T_{leaf}) and the deviation between the bud and leaf temperature (T_{bud}-T_{leaf}) during the leaf expansion of the 5th, 7th and 9th leaves (n=8) under the nine T_{bud}/T_{plant} treatments. Different letters within a row represent statistically significant differences ($P < 0.05$).

Temperatures		T_{bud}/T_{plant} treatment								
		18/18	22/18	26/18	18/22	22/22	26/22	18/26	22/26	26/26
5th	T_{leaf}	20.1e	20.4d	20.3de	21.8c	21.9c	21.9c	23.7a	23.6ab	23.4b
	T_{bud}-T_{leaf}	0.2c	1.6b	3.2a	-1.7d	0.3c	1.9b	-2.7e	-1.5d	0.0c
7th	T_{leaf}	19.5c	19.6c	19.7c	21.9b	21.9b	21.9b	23.6a	23.8a	23.8a
	T_{bud}-T_{leaf}	0.4c	2.4b	4.3a	-1.8d	0.3c	2.0b	-2.8e	-1.6d	0.0c
9th	T_{leaf}	19.4h	19.7g	19.9f	21.1e	21.9d	22.3c	23.4b	23.6b	23.8a
	T_{bud}-T_{leaf}	0.4d	2.3b	4.2a	-1.2f	0.2de	1.8c	-2.6g	-1.4f	-0.1e

Table S3. Estimated mean leaf expansion rate normalized for thermal time (LER_{dd}; 10^{-2} m^2 [^oC day]$^{-1}$) and leaf expansion duration in thermal time (LED_{dd}; ^oC days) of the 5th, 7th and 9th leaves (n=8) in relation to the mean leaf temperature (T_{leaf}; Table S2) and the deviation between the mean bud and mean leaf temperature (T_{bud}-T_{leaf}; Table S2) during the leaf expansion under the nine T_{bud}/T_{plant} treatments. The regression coefficient (b) for each factor (T_{leaf} or T_{bud}-T_{leaf}) represents the (positive or negative [-]) change in the dependent variable (i.e. LER_{dd} or LED_{dd}) per 1 ^oC increase in the factor under the assumption that the relation between the dependent variable and the factor is linear. Statistically significant effects with $P < 0.001$(***), $P < 0.01$(**), $P < 0.05$ (*) and non-statistically significant with $P > 0.05$ (n.s.).

Leaf #	Expansion trait	T_{bud}/T_{plant} treatment									General linear model		
											Regression coefficients (b)		Adjusted R^2
		18/18	22/18	26/18	18/22	22/22	26/22	18/26	22/26	26/26	T_{leaf}	T_{bud}-T_{leaf}	
5th	LER_{dd}	0.023	0.021	0.019	0.019	0.018	0.019	0.024	0.024	0.023	n.s.	n.s.	--
	LED_{dd}	421	381	390	534	498	472	479	457	423	n.s.	*** -16.8	0.30
7th	LER_{dd}	0.024	0.022	0.020	0.030	0.025	0.024	0.031	0.030	0.026	n.s.	***-0.002	0.70
	LED_{dd}	443	426	415	490	470	456	476	462	439	n.s.	*** -8.7	0.32
9th	LER_{dd}	0.022	0.020	0.017	0.031	0.027	0.023	0.032	0.029	0.023	n.s.	***-0.002	0.71
	LED_{dd}	447	435	417	469	451	467	459	440	430	n.s.	* -3.8	0.09

Chapter 5

Impact of light on leaf initiation: a matter of photosynthate availability in the apical bud?

Abstract

Radiation substantially affects leaf initiation rate (LIR), a key variable for plant growth, by influencing the heat budget and therefore the temperature of the shoot apical meristem. The photosynthetically active component of solar radiation (photosynthetic photon flux density; PPFD) is critical for plant growth and when at shade to moderate levels may also influence LIR via limited photosynthate availability. Cucumber and tomato plants were subjected to different PPFDs (2.5–13.2 mol m^{-2} d^{-1}) and then LIR, carbohydrate content and diel net CO_2 uptake of the apical bud were quantified. LIR showed saturating response to increasing PPFD in both species. In this PPFD range, LIR was reduced by 20% in cucumber and by 40% in tomato plants. Carbohydrate content and dark respiration were substantially reduced at low PPFD. LIR may be considered as an adaptive trait of plants to low light levels, which is likely to be determined by the local photosynthate availability. In tomato and cucumber plants, LIR can be markedly reduced at low PPFD in plant production systems at high latitudes, suggesting that models solely based on thermal time may not precisely predict LIR at low PPFD.

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Introduction

In higher plants, leaf initiation rate (LIR) is a measure of the number of leaves and phytomeres (i.e. plant modules consisting of a leaf, an internode and an axillary bud) initiated by the shoot apical meristem per unit of time and determines the time course of shoot morphogenesis and growth. LIR is highly dependent on meristem temperature ($T_{meristem}$; Jamieson *et al.* 1995; Granier and Tardieu 1998; Granier *et al.* 2002), which in turn depends on various environmental factors (Savvides *et al.* 2013). Thermal time (Trudgill *et al.* 2005) is widely used for modelling and predicting LIR in crop (e.g. sunflower; Granier and Tardieu 1998) and non-crop species (e.g. arabidopsis; Granier *et al.* 2002). Even though thermal time became 'common knowledge', there are also indications that it cannot always explain plant responses as temperature seems not the only factor influencing LIR. In dicot plants, other factors like water availability (Clough and Milthorpe 1975; Marc and Palmer 1976) and diel photosynthetic photon flux density (PPFD; Hussey 1963a; Newton 1963) were also reported as influential for LIR.

PPFD influences several developmental processes (e.g. root meristematic development; Freixes *et al.* 2002). However, PPFD effects on leaf initiation are still ambiguous. Numerous studies reported either positive (Hussey 1963a; Newton 1963; Pieters 1985; Marcelis 1993b; Cookson *et al.* 2005) or no relation of PPFD and LIR (Beinhart 1963; Heuvelink and Marcelis 1996). Species mobilize different strategies, and therefore, different physiological and morphological traits to adapt to their ever changing light environment (Valladares and Niinemets 2008). Therefore, the differences observed between studies may be the result of differences in the sensitivity of leaf initiation of different species to PPFD.

Besides these ecophysiological reasons, methodological differences may well be a reason for the deviation observed in earlier studies of LIR responses to PPFD. First, mostly air temperature (T_{air}) and to a lesser extent leaf temperature (T_{leaf}) are used as approximations of $T_{meristem}$. $T_{meristem}$ may deviate from T_{air} depending on other environmental factors, that are also influencing meristem heat budget, like radiation (Savvides *et al.* 2013). Therefore, $T_{meristem}$ may increase with increasing PPFD while T_{air} remains (or is regulated to be) constant. In addition, structural and functional differences between organs indicate different thermal

properties, and therefore, different organ temperatures even under identical environments (Geller and Smith 1982; Savvides *et al.* 2013). Consequently, using T_{air} or T_{leaf} as a rough estimate of $T_{meristem}$ may result in substantial misestimation of light effects on LIR. Second, it is usually assumed that the light quality (i.e. spectral distribution of photon flux density) is homogeneous when manipulating PPFD. Hence, it is often not quantified. However, PPFD manipulation may cause substantial changes in the light quality perceived by the plants depending on the methodology followed (e.g. the use of nettings that do not intercept all the wavelengths to an equal extent; Poorter *et al.* 2012). Light quality is highly influencing leaf development and functionality (Hogewoning *et al.* 2010; Savvides *et al.* 2012). Specifically, variation in red: far red ratio (Carabelli *et al.* 2007) and blue light fluence-rate under constant PPFD (Christophe *et al.* 2006) were reported as influential for leaf appearance and subsequent leaf expansion. Consequently, controversies between studies on the responses of LIR to PPFD may also be due to variation in light quality during experimentation. Third, the rates at which successive leaves appear (LAR; become visible to the naked eye) or unfold (LUR) are usually used as approximates of LIR. LIR is defined as the rate of the formation of successive projections (leaf primordia) on the meristem (dome; see Fleming *et al.* 1997) and it is the most appropriate indicator of the timing of leaf/phytomer formation. However, the need for laborious micro-stereoscopic (destructive) observations to estimate LIR led to the use of rates based on later visible leaf developmental stages (such as LAR and LUR). It was already shown that the early stages of leaf expansion (i.e. leaf initiation and leaf early growth) are correlated processes (Cookson *et al.* 2005). However, this well-defined correlation does not necessarily imply equality between LIR, LAR and LUR. Previous studies suggested equality on the long-term (Heuvelink and Marcelis 1996) but inequality on the short-term (e.g. early vegetative stage; Newton 1963). Consequently, it is still debatable whether LAR and/or LUR can be used as precise approximates of LIR under different PPFDs.

The response of LIR, LAR or LUR to PPFD may be related with the carbohydrate availability in the local tissue. Carbohydrates, despite being the substrate for growth, are also mediating the responses of several developmental and growth processes to light (Freixes *et al.* 2002; Moore *et al.* 2003). The meristem and the surrounding-folded developing leaves (i.e. apical bud) are considered as

sinks (i.e. imported carbohydrates are the main resource for growth and maintenance; Ho 1988). Sink-to-source transition in leaves begins shortly after unfolding (Turgeon 1989). The early stages of leaf expansion are strongly dependent on local carbohydrate availability and metabolism (Pantin *et al.* 2012). Therefore, it can be hypothesized that the PPFD responses of developmental and growth processes taking place within the apical bud are related with the local carbohydrate availability and utilization (metabolism). However, the relation between light and carbohydrate availability in the apical bud even though suggested (Hussey 1963b; Newton 1963; Marcelis 1993b) has not been yet investigated.

The rate at which leaves/phytomeres are initiated can be an adaptive trait of plants to changes in PPFD. The controversy between studies on the relation between LIR and light strengthens the necessity to further unravel the relation between LIR and PPFD. In this study we aim (1) to determine and quantify the response of LIR to (changes in) PPFD in cucumber (*Cucumis sativus* L.) and tomato (*Solanum lycopersicum* L.) while explicitly taking into account the differential impact of radiation on $T_{meristem}$ and quantifying light quality (2) to relate LIR, LAR and LUR under different light levels, (3) to investigate whether PPFD substantially influences the carbon status and utilization of the apical bud. Cucumber and tomato plants were used as model plant systems as they have shown similarities on the response of LIR to PPFD (Hussey 1963a; Newton 1963) but also discrepancies on the response of LAR to carbohydrate availability (Marcelis 1993b; Heuvelink and Marcelis 1996).

Materials and methods

Plant material and growth conditions

Cucumber (*Cucumis sativus* L. cv. Venice, Rijk Zwaan) and tomato (*Solanum lycopersicum* L. cv. Cappricia, Rijk Zwaan) plants were germinated in wet rockwool and grown until the cotyledons had unfolded (i.e. when the first true leaf was visible) in a climate room at 21.5 ^{o}C T_{air}, 70% RH, ~380μmol mol^{-1} [CO_2]. The plants were illuminated by SON-T lamps (MASTER GreenPower CGT 400W E40 1SL;

Royal Philips Electronics N.V., Amsterdam, The Netherlands) at a 16 h photoperiod. The lamps were isolated from the climate room by a glass ceiling which enabled the separate convective cooling of the lamps. Below the glass ceiling an energy screen (OLS60; *AB Ludvig Svensson*, Kinna, Sweden) was added to sustain the homogeneous distribution of light within the climate room and reduce the thermal radiation emission by the lamps into the climate room. The light intensity was 230 μmol m^{-2} s^{-1} resulting in 13.2 mol m^{-2} d^{-1} PPFD. After emergence the plants were watered with nutrient solution (EC = 2 dS m^{-1}, pH = 5.0 - 5.5) in an ebb and flood irrigation system.

Light treatments

When the first leaf was visible, the plants were transferred into four different light intensities 230 (control), 113, 85 and 44 μmol m^{-2} s^{-1}. The different light treatments were carried out one after the other in the same climate room and both the plant species were treated simultaneously. The lower light intensities were created by transversely adding more energy screen layers. In descending order, the second and third PPFD were created with the addition of a second and a third layer of the same energy screen (OLS60) respectively. The fourth light intensity was created with the addition of a third layer of an energy screen with different pattern of openings (XLS 16 F firebreak; *AB Ludvig Svensson*). The photoperiod was kept at 16h resulting in 13.2, 6.5, 4.9 and 2.5 mol m^{-2} d^{-1} PPFD (Table 1).

Table 1. Environmental conditions and diel average meristem temperatures ($T_{meristem}$) in the treatments. Values of $T_{meristem}$ are the means of measurements on eight plants ± s.e.

Light intensity (μmol m^{-2} s^{-1})	PPFD (mol m^{-2} d^{-1})	T_{air} (°C)/RH (%)		$T_{meristem}$ (°C) (±s.e.)	
		Cucumber	Tomato	Cucumber	Tomato
44	2.5	21.5/70	21.8/70	20.6 (±0.1)	21.5 (±0.1)
85	4.9	21.8/70	21.8/70	20.4 (±0.3)	21.7 (±0.1)
113	6.5	21.8/70	21.8/70	20.6 (±0.3)	22.1 (±0.2)
229	13.2	21.5/70	21.8/70	20.5 (±0.1)	21.7 (±0.2)

The spatial distribution of light quality and intensity were measured at plant level by a spectroradiometer (USB2000, Ocean Optics, Duiven, The Netherlands). The phytochrome photostationary state (PSS; Sager *et al.* 1988) was estimated using a

custom-made software (built using LabVIEW 8.6.1, National Instruments, Austin, TX, USA). The light quality was homogeneous under the different PPFD treatments (Fig. 1). The PSS value was ~0.86 and the percentage of blue light (400-500 nm) was ~5% in all the PPFD treatments.

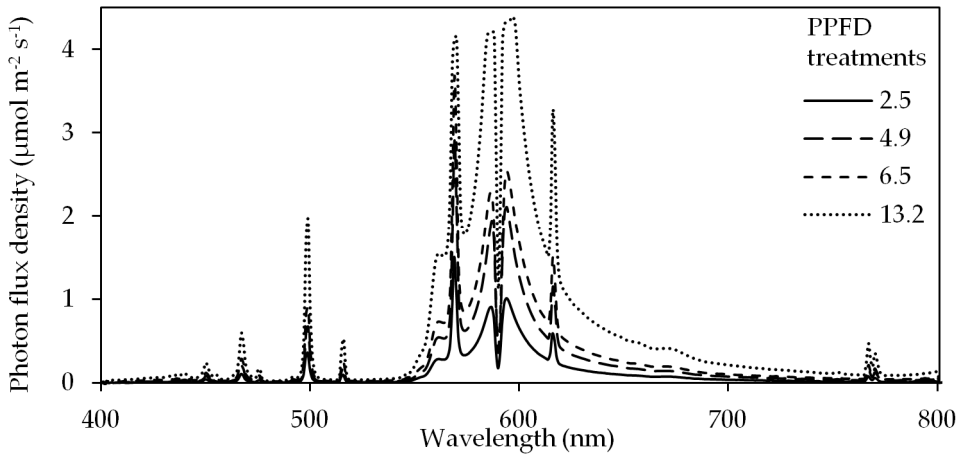

Fig. 1. Spectral distribution of the photon flux density in the four photosynthetic photon flux density (PPFD) treatments.

The treatments ended 36 days after plants were moved in the treatments. 22 days after the treatments started, 5 plants were transferred from the highest to the lowest and from the lowest to the highest PPFD until day 36 to test the response of LIR, LAR and LUR to a change in PPFD (swap treatments).

Plant density was set at 14 plants per m^2 at the beginning of the treatments and plant spacing was increased during the experiment to avoid inter-plant shading and maintain the same light environment within the light treatments. Side shoots were removed when they reached the length of 2cm to maintain single shoot plants and flowers were also removed when out of the apical bud to keep both species vegetative.

T_{air}, RH and $T_{meristem}$ during the treatments were measured as described by Savvides *et al.* (2013). $T_{meristem}$ was measured by k-type fine-wire thermocouples and T_{air} and RH were monitored by a temperature/humidity sensor (1400-104; Li-Cor Inc., Linkoln, NE, USA) every 1 min and then averaged per diel cycle. The $T_{meristem}$ was measured on 8 plants per species per treatment (Table 1). $T_{meristem}$ was used to

estimate thermal time and distinguish between the effects of radiant environment through changes in $T_{meristem}$ and the effects of PPFD on the number of leaves initiated, appeared and unfolded.
Thermal time (in degree (^{o}C) - days) was estimated based on:

$$Thermal\ time = \sum_{n=1}^{k} [(Diel\ T_{meristem})_n - T_{base}] \qquad \textbf{(Eqn. 1)}$$

Diel $T_{meristem}$ is the diel integration (mean) of $T_{meristem}$ while T_{base} is the base temperature at which cessation of the developmental process occurs (Trudgill *et al.* 2005). k is the number of days after the start of the treatments at which the developmental measurements occurred. The T_{base} used for cucumber was 10^{o}C (Marcelis 1994) and for tomato 8^{o}C (estimated based on a published relation between leaf appearance rate and temperature; Adams *et al.* 2001).

Plant measurements
Plant development: The number of leaves initiated per unit of time (i.e. the total number of leaves present on a plant per unit of time) was quantified by adding the visible and not visible (to the naked eye) leaves on a plant at 0, 22 and 36 days after the start of the treatments. The visible leaves - were counted by eye while the very young and invisible (leaf primordia) in the apical bud were quantified by dissecting the apical bud under a microstereoscope (Wild M7 S, Heerbrugg, Switzerland; 60× – 310×). The latest initiated leaf primordium was defined as the latest formed projection that was visible at the side of the meristem (dome). The number of leaves initiated per unit of (calendar) time (days) was defined as LIR. The number of leaves initiated per unit of thermal time (degree (^{o}C) - days) was defined as LIR_{DD}.

The number of appeared and unfolded leaves was quantified every two days from the beginning until the end of the treatments. A leaf was considered appeared when its length (petiole + lamina length) reached 4cm. A leaf was considered unfolded when its length reached 10cm. Measurements on plant development were conducted on 10 plants per species in continuous treatments and on 5 plants per species in swap treatments.

Apical bud carbohydrate contents: After the 7th leaf had unfolded in the lowest and highest PPFD, apical buds were detached at the end of day and the end of night for soluble carbohydrates (sugars) and starch analysis. The soluble carbohydrates that were monitored were fructose, glucose and sucrose, stachyose and raffinose. The samples were inserted in vials and were flash-frozen in liquid nitrogen. After freeze-drying (Modulyo®; Edwards, Crawley, UK), the samples were powdered in a mechanical grinder. A 15mg portion of each sample was placed for 20min in 5ml 80% ethanol at 80°C for sugar extraction. The supernatants were vacuum-dried (SpeedVac SPD 2010; Thermo Fisher Scientific Inc., Asheville, NY, USA) and re-suspended in 1ml Milli-Q water. To assure that carbohydrates were fully re-suspended in the aqueous phase, samples were placed in an ultrasonicator (Branson 2200; Branson Equipment Co., Shelton, CT, USA) for 10 minutes. After the re-suspension, samples were diluted 10 times (0.1 ml sample – 0.9 ml Milli-Q water). The soluble sugars were quantified using high-pressure anion exchange chromatography (HPAEC; ICS5000; Dionex, Sunnyvale, CA, USA). The separation was performed at 25°C in an anion exchange column (250x2mm; CarboPac® PA1; Dionex) with NaOH (100 mM) as mobile phase degassed with helium and pressurized using a pump (Bio LC; Dionex) at a flow rate of 0.25 ml min^{-1}. Detection was carried out by pulsed amperometry (PAD 2; Dionex). The chromatograms were analyzed using Chromeleon 7.0 software (Dionex) and the components (glucose, fructose, sucrose, raffinose and stachyose) were quantified.

For starch content quantification the pellets, after being washed three times with 80% ethanol, were vacuum-dried. Starch was enzymatically converted to glucose by thermostable α-amylase (Serva 13452) in water at 90°C and subsequently by amyloglucosidase (Fluka 10115) in 50mM citrate buffer with pH = 4.6 at 60°C. The samples were analyzed for glucose on an HPLC Dionex system (GS 50 pump and PED 2 electrochemical detector) equipped with a CarboPac PA1 (250x2mm) column and eluted with 100 mM NaOH and 12.5 mM sodium acetate. Chromatograms were analyzed using Chromeleon 6.4 software (Dionex). Carbohydrate measurements were conducted on 7 plants per species per time point (day and night) and normalized (divided) for apical bud dry weight.

Apical bud gas exchange measurements: After the 7th leaf had unfolded diel net CO_2 uptake (net photosynthesis during the day and dark respiration during the night) of the apical bud was measured, only in the lowest and highest PPFD

treatments, using the gas exchange method described by Savvides *et al.* (2013). The apical bud was enclosed within a transparent spherical chamber connected to the sample tubing of LI-6400 portable gas exchange system (Li-Cor Inc., Linkoln, NE, USA). During the measurements, the microclimatic conditions within the sphere were maintained similar to the ambient environment. $T_{meristem}$ was continuously monitored during the measurements to assure no effects of temperature on bud net CO_2 uptake. Gas exchange measurements were conducted on 6 plants per species and normalized (divided) for apical bud DW.

Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate statistically significant effects of PPFD on LIR and LIR_{DD}, and significant differences between treatments' means were evaluated with *post hoc* Tukey's honestly significant difference (HSD) multiple comparison tests ($P < 0.05$). Two-way ANOVA was used to evaluate statistically significant effects of PPFD and time (day and night) on apical bud carbohydrate contents ($P < 0.05$). Statistical analyses were carried out with the R software (R 3.0.1; The R Project for Statistical Computing, Vienna, Austria).

Results

The response of leaf initiation to light

The initiation of new leaves/phytomeres substantially decelerated with decreasing PPFD in tomato and cucumber plants within the PPFD range studied (Fig. 2). LIR (Fig. 2*a*) and LIR_{DD} (LIR normalised for thermal time; Fig. 2*b*) showed saturating response to increasing PPFD in both tomato and cucumber plants. The similarity between LIR (Fig. 2*a*) and LIR_{DD} (Fig. 2*b*) as a function of PPFD is explained by the absence of substantial differences in $T_{meristem}$ across the PPFD treatments (Table 1). However, tomato plants showed, in all treatments, higher $T_{meristem}$ (~ 1 ^{o}C) than cucumber plants (Table 1).

Despite that in both species LIR_{DD} did show saturating response to PPFD, the relative (to the highest PPFD) reduction in LIR_{DD} within the PPFD range

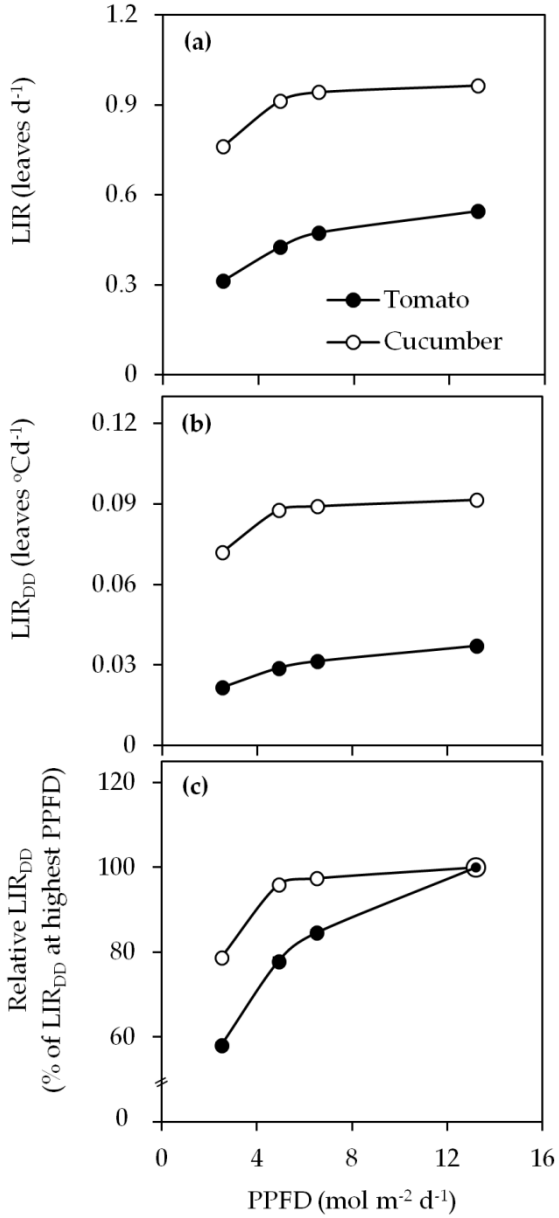

Fig. 2. The saturating response of (*a*) leaf initiation rate (LIR), (*b*) leaf initiation rate normalized with thermal time (LIR_{DD}) and (*c*) relative LIR_{DD} (percentage of LIR_{DD} at the highest photosynthetic photon flux density (PPFD)) to diel PPFD in tomato (closed symbols) and cucumber plants (open symbols). Values are the means of measurements on 10 plants ± s.e. (error bars are smaller than the data points).

studied was higher in tomato (40%) than in cucumber plants (20%; Fig. 2*c*). In cucumber plants LIR substantially reduced ($P < 0.001$) only at the lowest PPFD (2.5 mol m^{-2} d^{-1}), while in tomato plants, LIR significantly decreased ($P < 0.001$) with decreasing PPFD and the drop was larger the lower the PPFD (Fig. 2*c*). More leaves were initiated in cucumber plants (higher LIR; Fig. 2*a*) than in tomato plants in the course of time (lower LIR; Fig. 2*a*). Although, from the start of the treatments onwards the number of leaves initiated was linearly related to time in both species (Fig. 3*a, b*).

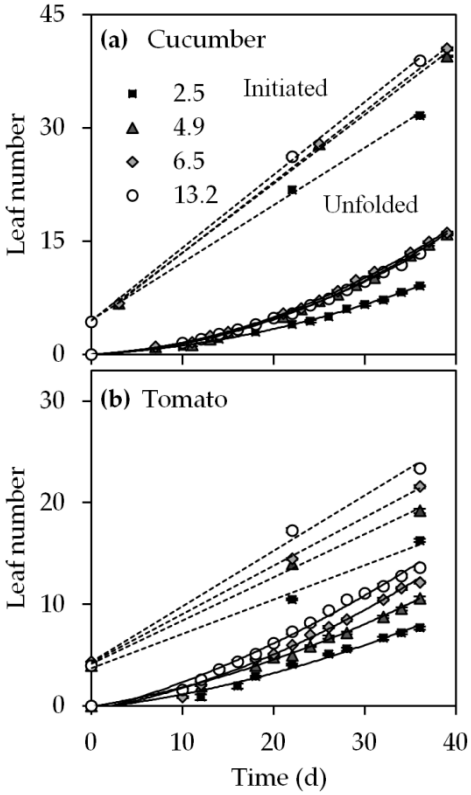

Fig. 3. Time course of the number of leaves initiated (dashed lines) and unfolded (length 10cm; solid lines) in cucumber (a) and tomato plants (b) grown under four diel photosynthetic photon flux densities (2.5, 4.9, 6.5 and 13.2 mol m^{-2} d^{-1}). Values are the means of measurements on 10 plants ± s.e. (error bars are smaller than the data points).

Shifting plants between the lowest and highest PPFDs (2.5 and 13.2 mol m^{-2} d^{-1}) and vice versa resulted in disruption of the linear increase and rapid (within days) changes in the rates of leaf initiation both in cucumber (Fig. 4*a*) and tomato plants (Fig. 4*b*). The rate at which leaves initiated after the swap to the highest PPFD increased and it tended to be equal to the rates observed on the plants continuously grown under the highest PPFD in both species (Fig. 4*a, b*); similarly

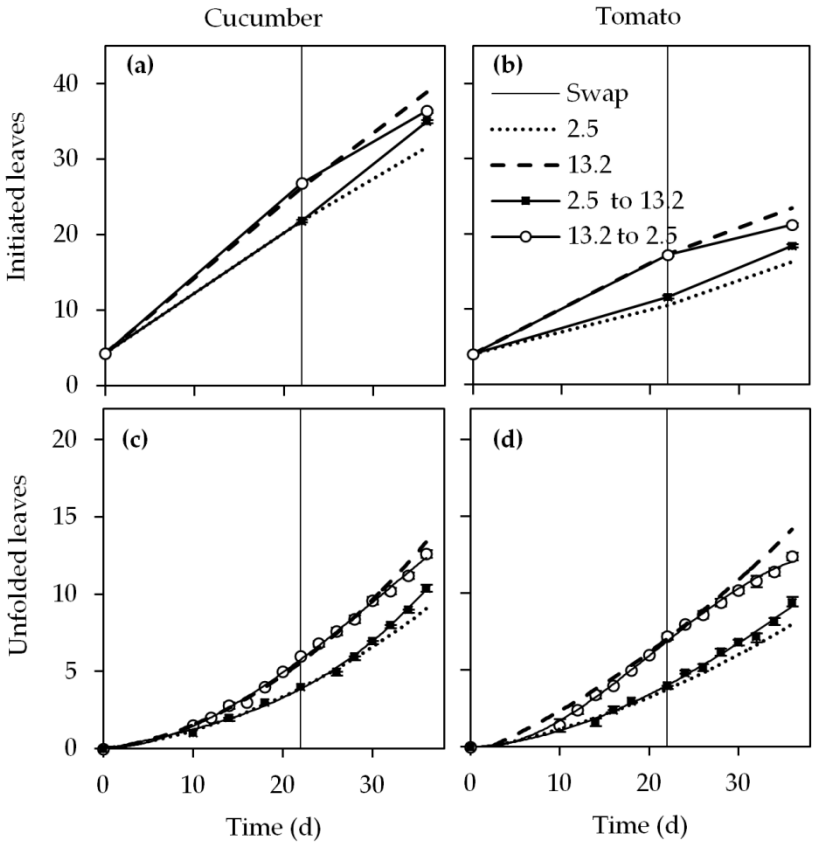

Fig. 4. The alteration in the number of leaves initiated (upper) and unfolded (lower) as a function of time before and after the cucumber (left) and tomato plants (right) were transferred (vertical solid line) from the highest to the lowest (13.2 to 2.5 mol m^{-2} d^{-1} (open circles)) and from the lowest to the highest PPFD (2.5 to 13.2 mol m^{-2} d^{-1} (closed circles)) in comparison to the continuous treatments (13.2 mol m^{-2} d^{-1} (dashed line) and 2.5 mol m^{-2} d^{-1} (dotted line)). Values are the means of measurements on 5 plants ± s.e. (error bars are smaller than the data points).

LIR decreased after swapping to the lowest PPFD and it was comparable to that of plants continuously grown at the lowest PPFD (Fig. 4*a, b*).

Comparison of the number of initiated, appeared and unfolded leaves

In contrast to the linear increase of initiated leaves in the course of time (Fig. 3*a, b*), a delay was observed on the unfolding (Fig. 3*a, b*) and similarly on the appearance (data not shown) of leaves in both species. After the first leaf had unfolded, the number of leaves unfolded increased curvy-linearly in both species (Fig. 3*a, b*). Therefore, LIR was higher than LUR at the early plant development and LUR was actually increasing towards LIR with plant age. In tomato plants, LUR became similar to LIR faster than in cucumber plants. PPFD had similar effects on LIR and LUR. The rate at which leaves unfolded decreased with decreasing PPFD in tomato (Fig. 3*b*), while it only substantially decreased at the lowest PPFD in cucumber plants (Fig. 3*a*).

Shifting plants between the lowest and the highest PPFD (2.5 to 13.2 mol m^{-2} d^{-1}) and vice versa resulted in changes also in the number of leaves unfolded per unit of time both in cucumber (Fig. 4*c*) and tomato plants (Fig. 4*d*). Alterations in LUR with changes in PPFD followed the alterations in LIR in both species (Fig. 4).

Carbohydrate contents in the apical bud

Soluble carbohydrates and starch contents in the apical bud were significantly higher ($P < 0.001$) at the highest than at the lowest PPFD in both species and these differences were larger in tomato than in cucumber plants (Table 2). Soluble carbohydrates content in the apical bud was significantly higher ($P < 0.001$) at the end of the day than at the end of the night in cucumber plants but not in tomato plants ($P = 0.84$). Starch content in the apical bud was significantly higher ($P < 0.001$) at the end of the day than at the end of the night in both species. Interaction between PPFD and day/night was observed on the starch content only in tomato plants ($P < 0.001$). In cucumber plants, the starch content was almost two-fold higher at the end of the day than at the end of the night in the apical bud at both the lowest and highest PPFD. In tomato plants, starch content was more than two-fold higher at the end of the day than at the end of the night in the apical bud at

high PPFD while the difference in starch content between the end of the day and the end of the night was much lower at low PPFD.

Table 2. Soluble carbohydrates and starch content in cucumber and tomato apical buds at the end of day and the end of night at low (2.5 mol m^{-2} d^{-1}) and high diel photosynthetic flux density (PPFD; 13.2 mol m^{-2} d^{-1}). Values are the means of measurements on seven plants. The means were tested with two-way analysis of variance (ANOVA) for significant effects of PPFD, day/night and the interaction between PPFD and day/night (PPFD × day/night) on soluble carbohydrates and starch contents. Significant differences are indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., non-significant; n.d., not detected.

Carbohydrates (mg g^{-1} DW)	PPFD (mol m^{-2} d^{-1})				Two-way ANOVA		
	2.5		13.2		PPFD	Day/ night	PPFD × day/night
	Day	Night	Day	Night			
			Cucumber				
Soluble carbohydrates	16.5	11.9	19.3	15.7	***	***	n.s.
Glucose	1.3	1.3	1.8	2.5	***	*	*
Fructose	0.8	0.8	0.9	1.1	*	n.s.	n.s.
Sucrose	9.4	6.8	11.2	7.7	**	***	n.s.
Raffinose	3.9	2.2	4.5	3.6	***	***	n.s.
Stachyose	1.0	0.8	0.9	0.9	n.s.	**	n.s.
Starch	15.7	8.3	30.6	19.3	***	***	n.s.
			Tomato				
Soluble carbohydrates	16.0	16.7	37.2	34.9	***	n.s.	n.s.
Glucose	3.5	4.1	13.9	14.6	***	n.s.	n.s.
Fructose	1.8	2.4	6.1	4.8	***	n.s.	n.s.
Sucrose	10.5	10.0	16.8	15.2	***	n.s.	n.s.
Raffinose	n.d.	n.d.	n.d.	n.d.	–	–	–
Stachyose	0.3	0.1	0.4	0.3	***	***	n.s.
Starch	12.6	7.6	72.3	28.1	***	***	***

The soluble carbohydrates sucrose, raffinose, glucose, fructose and stachyose were detected in descending order of content in the apical buds of cucumber plants. A similar order was measured in tomato, except that no raffinose was detected (Table 2). The contents of the individual soluble carbohydrates were significantly lower at the lowest than at the highest PPFD in both species except for

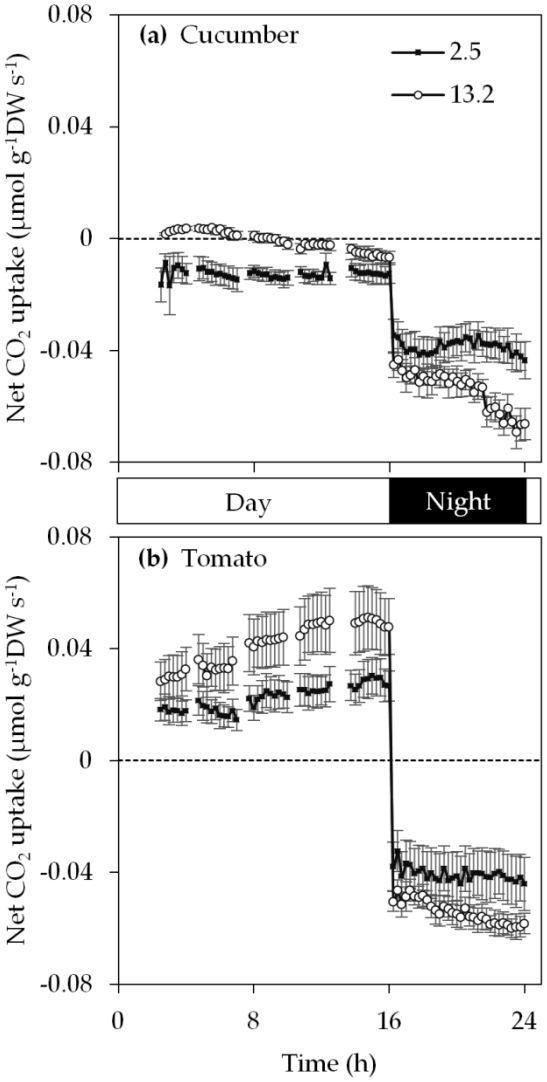

Fig. 5. The diel pattern of the net CO_2 uptake of the apical bud in cucumber (*a*) and tomato plants (*b*) grown at high (13.2 mol m^{-2} d^{-1} (open symbols)) and low photosynthetic photon flux density (PPFD; 2.5 mol m^{-2} d^{-1} (closed symbols)). Values are the means of measurements on six plants ± s.e.

stachyose in cucumber plants (P = 0.77). In cucumber plants, the contents of the individual soluble carbohydrates were significantly higher at the end of the day than at the end of the night except for fructose (P = 0.39) while in tomato the

contents of the individual soluble carbohydrates were not significantly different between the end of the day and the end of the night except for stachyose ($P < 0.001$). Interaction between PPFD and day/night was observed only on glucose content in cucumber plants ($P < 0.05$).

Diel pattern of apical bud net CO_2 uptake

The diel net CO_2 uptake of the apical bud was substantially altered by PPFD in both cucumber and tomato plants. Both the apical bud net photosynthesis during the day and the apical bud respiration rate during the night were higher at the highest PPFD in comparison to the lowest PPFD in both species (Fig. 5). In cucumber plants, net photosynthesis was almost zero at the highest PPFD and negative at the lowest PPFD during the day (Fig. 5*a*). In tomato plants, the net photosynthesis was higher than in cucumber plants and positive at both PPFDs (Fig. 5*b*). The order of magnitude of dark respiration was similar between cucumber and tomato plants.

Discussion

Low light levels cause substantial deceleration of leaf initiation in cucumber and tomato plants

In this study we clearly showed that, LIR substantially decreased in tomato and cucumber plants below a certain light level (6.5 mol m^{-2} d^{-1}; Fig. 2). This effect was evidently not related to $T_{meristem}$ as it was fairly constant among the light treatments (Table 1). PPFDs below 6.5 mol m^{-2} d^{-1} can be found in nature (e.g. forest floors; Chazdon and Fetcher 1983), in protected cultivation (especially during winter at high latitudes; Marcelis and Gijzen 1998) as well as in controlled experimentation sites (e.g. climate rooms). This implies that under these circumstances predictions of the number of leaves/phytomeres initiated in the course of time based on models, which are exclusively based on thermal time, may be prone to substantial errors.

In both species LIR showed a saturating response to PPFD, although LIR in cucumber plants was two-fold higher than in tomato plants (Fig. 2). Saturating

response of LIR to PPFD from low to intermediate light levels was also shown in previous studies (Hussey 1963a; Newton 1963). Therefore, above a certain PPFD threshold, no response of LIR to PPFD is expected. This may partly explain why in certain studies no reduction in LIR was observed (e.g. sweet pepper; Heuvelink and Marcelis 1996). The difference observed on the reduction of the relative LIR_{DD} between tomato (40%) and cucumber plants (20%) within the same PPFD range (Fig. 2*c*) supports that the sensitivity of the process of leaf initiation to PPFD may be species-specific. Accordingly, leaf initiation may be unequally influenced by PPFD when comparing between different species.

Production of less phytomeres under shade was also observed in other studies (Hussey 1963a; Newton 1963; Chenu *et al.* 2005; Cookson *et al.* 2005; Christophe *et al.* 2006). We here showed that except the long-term responses to PPFD (continuous treatments; Fig. 2); LIR closely followed the short-term changes in PPFD (swap treatments; Fig. 4*a, b*). The rapid (within days) and consistent changes in the rate at which these plant species form new sinks (e.g. young phytomeres) suggests a fast and well-coordinated adaptation of plant growth to the available resources. Plant adaptation to the available resources is a prerequisite for starvation avoidance and plant survival (Smith and Stitt 2007). Plants tolerate shade environments by maximizing their light capture and minimizing their respiration costs (Givnish 1988). Shade environments are characterized by reduced PPFD (and reduced blue light) and low red: far-red ratio both highly influential for plant morphogenesis (Stuefer and Huber 1998; Kozuka *et al.* 2005). Low red: far-red ratio triggers shade avoidance responses (e.g. plant elongation) and generates a competitive plant profile at low light availability (Franklin and Whitelam 2005). In present experiments the other component of a shade environment, the low PPFD became limiting for the process of leaf/phytomer initiation. The lower rates of leaf/phytomer production may be sustaining carbon economy by reducing the carbon expenses into new organs. The low rate of leaf/phytomer production is also sustaining a lower rate of plant self-shading. In this way a plant may achieve lower carbon expenditure to new organs and more efficient light capture by the existent leaves. In combination with enhanced plant elongation because of the low red: far-red ratio, lower LIR would potentially enhance plant competitive capacity in natural shade environments. Consequently, reduction in LIR may be considered as

one of the plant responses enhancing shade tolerance, plant competitive capacity and sustaining plant survival.

Leaf initiation rates are correlated but are not always equal with leaf unfolding rates across different light levels

The timing of the initiation of a new leaf (i.e. primordium) strongly depends on the expansion of the latest formed primordia (Golz 2006). Cookson et al. (2005) suggested that leaf initiation and initial relative leaf expansion rates are correlated across different light environments and genotypes in *Arabidopsis thaliana* (L.). Consequently, leaf initiation and early leaf expansion can be considered as inter-connected processes. We here showed that in young tomato and cucumber plants the rates in which successive leaves had unfolded followed the rates in which successive leaves were initiated across the PPFD treatments (Fig. 3). In cucumber especially (although less in tomato), even though a distinct relationship appeared between LIR and LUR across PPFDs (Fig. 3), LUR was much lower than LIR and increased with plant age. This explains the increasing number of leaves in the apical bud observed in the course of time as more leaves were initially initiated than unfolded away from the apical bud. Even though LUR was increasing with plant age in young plants, shifting plants between the lowest and the highest PPFD and vice versa resulted in, similar to LIR, alterations in LUR (Fig. 4) indicating that LUR is following LIR even in varying light environments. The inequality between LIR and LUR in young cucumber plants indicates that LUR cannot always approximate LIR. For example, LIR would be underestimated if LUR was quantified as its approximation. Therefore, the assumption of constant LUR (i.e. linear increase of the number of leaves appeared or unfolded) in such species during the early plant development is not valid. On top of that, the differences observed between young tomato and cucumber plants suggest that the relation between LIR and LUR can be species-specific. Overall, LUR may be used as qualitative but not quantitative approximation of LIR when investigating PPFD effects on LIR.

The number of leaves accumulated in the apical bud is not expected to indefinitely increase in cucumber plants. Therefore, equality between LIR and LUR is expected in later stages (Marcelis 1993b; Turc and Lecoeur 1997). The lower LUR in comparison to LIR indicates that the duration from initiation to unfolding

increases implying that leaf expansion rate decreases with leaf number during the early plant life. Such differences between successive leaves in the early plant development were also observed in other species (*Pisum sativum* L.; Turc and Lecoeur 1997; *Sorghum bicolor* L.; Lafarge and Tardieu 2002; *Helianthus annuus* L.; Dosio *et al.* 2003). This phenomenon seems to be ontogenetic (Dosio *et al.* 2003) and genetically programmed (i.e. heteroblasty; Kerstetter and Poethig 1998).

Is the decrease in LIR an adaptive trait to low light levels regulated by the carbon availability in the local tissue?

A reduction in LIR and in the subsequent early leaf expansion rate (look at LUR) may be an adaptive trait to low light levels towards preserving carbon economy and therefore avoiding starvation. Is this reduction though regulated directly by the local carbon availability?

Soluble carbohydrates (sugars) are considered as substrate for tissue growth and maintenance but also as signal inducers for plant development (Farrar *et al.* 2000; Eveland and Jackson 2012). The effects of PPFD on plant development may then be mediated by the carbon status of the local tissue. For example, the reduction in root elongation and branching induced by reduced PPFD was related with the reduction of hexose (glucose and fructose) concentrations in the apical and sub-apical regions of the roots (Freixes *et al.* 2002). Previous studies, in which sink-source relations were altered on whole plant scale, suggested a linkage between LIR (and LAR) and photosynthate availability. For example, LAR increased in generative cucumber plants when fruit number (sinks number) decreased (Marcelis 1993b); LIR increased when young leaves (sinks) were removed in tomato seedlings (Hussey 1963b); LIR decreased when cotyledons (the main source of carbon) were removed in tomato seedlings (Hussey 1963b). Here we showed a significant reduction in soluble carbohydrates and starch contents of the apical bud with a reduction in PPFD in both species (Table 2). In tomato plants this reduction was larger than in cucumber plants (Table 2). Tomato plants also showed larger LIR (and LUR) reduction when compared to cucumber plants. In addition, in both species the apical buds had lower dark respiration rates at lower PPFD (Fig. 5). The reductive effects of low PPFD on dark respiration may be mediated by the low photosynthate availability (Noguchi 2005) suggesting that at these PPFD levels, carbohydrate status is limiting local metabolic activities and

therefore growth processes within the apical bud. This is in accordance with the notion that early leaf growth processes are highly determined by photosynthate availability and metabolics (Pantin *et al.* 2011; Pantin *et al.* 2012).

Here we have shown that low PPFD, most probably through local photosynthate availability, largely reduces LIR and LUR. Photosynthate availability in the apical bud may be limiting for LIR with decreasing PPFD but this does not necessarily exclude the involvement of other light signal mediators in the process of leaf initiation. For example, it was previously reported that blue light fluence-rate, which is also increasing with increasing PPFD, may act as antagonist to the enhancing effect of PPFD on leaf appearance and leaf expansion in *Trifolium repens* L. Accordingly, the reducing effect of photosynthate availability may be partly counterbalanced by spectral-specific light signals. The involvement of photoreceptors in the process of leaf initiation was previously suggested (Carabelli *et al.* 2007; Yoshida *et al.* 2011). This indicates the necessity for further research on the response of leaf initiation to the natural light environment and integration of the underlying mechanisms involved in this response.

Conclusions and future perspectives

Leaf initiation substantially decelerates under low light levels in tomato and cucumber plants. This highlights the necessity for considering, besides meristem temperature, also PPFD as a largely influential factor for LIR in future studies and applications (e.g. models). LIR and subsequent early leaf expansion seem to be traits for plant adaptation to shade environments. The correlation of photosynthate availability in the local tissue with PPFD suggests the involvement of photosynthate availability in the response of LIR and early leaf expansion to PPFD.

Further research is needed towards 1) disentangling the effects of the different components of the shade environment (e.g. reduced PPFD, red: far-red and blue light fluence) on leaf initiation and 2) unravelling but also integrating the potential different mechanisms relating light environment to the process of leaf initiation.

Acknowledgments

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Chapter 6

General Discussion

The central aim of this thesis was the linkage of leaf (phytomer) initiation rate (LIR) at the shoot apical meristem (SAM) to the aerial environment. Using two major horticultural crop species, *Cucumis sativus* L. (cucumber) and *Solanum lycopersicum* L. (tomato), it has been shown that air temperature (T_{air}) is a major component-factor (Chapter 2) but not the only one in the linkage between LIR and the aerial environment (Chapter 2, 3, 5).

The temperature-responses of LIR were solely attributed to apical bud temperature (T_{bud}) even when the latter was greatly altered from the temperature of the rest of the plant (T_{plant}; Chapter 3). The within-bud or SAM temperature ($T_{meristem}$) was not only a function of T_{air} but a function of different environmental variables (T_{air}, net radiation, vapour pressure deficit and wind speed) and heat exchange-related variables of the apical bud (i.e. transpiration and structure; Chapter 2). In addition, LIR was reduced at low light levels (Chapter 5) indicating that LIR, except being a function of temperature, is also a function of photosynthetic photon flux density (PPFD).

Integrating from apical bud to plant level, the sole dependence of LIR to T_{bud} yielded important changes in plant phenotype when cucumber plants were subjected to altered T_{bud} (Chapter 4).

In this Chapter the findings of this thesis are brought together and discussed in a wide-ranging approach from a microclimatic to a physiological point of view. In addition, the implications of these findings in the study of plant ecophysiology, plant production systems and plant growth modeling are briefly discussed.

6.1. Linking leaf initiation to the aerial environment

6.1.1. Air temperature is not the whole story: a microclimatic approach

T_{air} is a main component of the sensible heat exchange between the apical bud and its environment and by that influences the heat budget and consequently $T_{meristem}$. However, sensible heat exchange is not the only heat exchange process that influences $T_{meristem}$ or any plant organ temperature in general (Fig. 1; Jones 1992; Lambers *et al.* 2008; Nobel 2009). Accordingly, it has to be emphasized that the temperature of the plant organ and not T_{air} influences physiological processes in the plant organ. Even though this is widely-known, for practical reasons T_{air} is often used as an approximation of plant organ temperatures. Using T_{air} as a proxy for plant organ temperature is actually not always wrong as under certain circumstances T_{air} may be very close to plant organ temperature (see Chapter 2). The proximity of T_{air} to plant organ temperature but also the degree of deviation between the two depends on other environmental components and plant organ traits (Fig. 1; Lambers *et al.* 2008).

Previous research showed that extreme environments yield large meristem-air temperature differences ($T_{meristem}$-T_{air}; Smith 1974; Wilson *et al.* 1987). In this study, it has been shown that $T_{meristem}$ may largely deviate from T_{air} even under moderate conditions (Chapter 2). Under the studied environments, $T_{meristem}$-T_{air} in tomato plants ranged from -2.6 to 3.8 ^{o}C, while in cucumber plants it ranged from -4.1 to 3.0 ^{o}C when T_{air} was kept at ~20 ^{o}C. Net radiation and wind speed were important determinants of $T_{meristem}$ (Fig. 1) and consequently of $T_{meristem}$-T_{air} in tomato plants while in cucumber plants also vapour pressure deficit was an important determinant (Fig. 1; Chapter 2). Consequently, $T_{meristem}$ is not only a function of T_{air} but a result of all the environmental variables affecting the heat exchange between the meristem and the environment, such as the air temperature, wind speed, radiation and vapour pressure deficit (Fig. 1).

Interestingly, the two species did not experience the same $T_{meristem}$ when subjected to the same environments. The observed differences in $T_{meristem}$ between the two species studied were due to differences in apical bud structure and transpiration (Chapter 2). Small or even large visible structural differences and

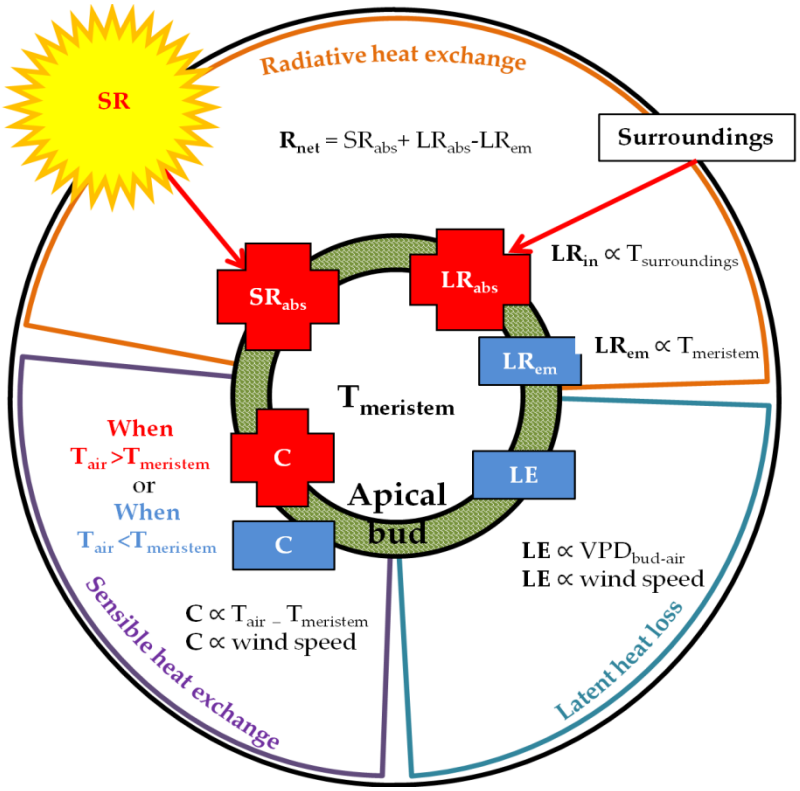

Fig. 1. Schematic representation of the heat exchange processes (the determinants of meristem heat budget and temperature) taking place between the shoot apical meristem (SAM) and the environment. The outer circle represents the external environment and the green circle represents the apical bud, the immediate environment of the SAM. The red plus (+) and the blue minus (-) signs represent the heat input (influx) to- and the heat output (efflux) from the SAM respectively. Instantaneous increase in influx results in increasing $T_{meristem}$ which in turns result in increase in efflux until the influx and efflux come into equilibrium. Instantaneous increase in efflux results in decreasing $T_{meristem}$ which in turns result in increase in influx until equilibrium. The net radiative influx (R_{net}; net radiation) is the sum of the absorbed shortwave- (SR_{abs}), longwave- (LR_{abs}) and emitted longwave radiation (LR_{em}). Latent heat loss by transpiration (LE) is a function of the vapour pressure difference between the organ and the air and wind speed. Sensible heat can be considered as influx or efflux depending on whether organ temperature is lower or higher than air temperature respectively. Sensible influx or efflux (C) is analogous to the difference between organ and air temperature and wind speed. All the heat exchange processes are limited by heat exchange-related organ traits (e.g. structure and transpiration capacity; Chapter 2).

'invisible' functional differences between plant species are usually neglected when relating organ physiology to the environment especially for plant organs other than planar leaves. The interspecific differences in structure and transpiration of the buds observed here were more than enough to cause substantial differences in $T_{meristem}$ between the two plant species studied. Consequently, $T_{meristem}$ should be treated as species-specific trait highly reliant on the environment.

In this study, when it comes to physiological temperature effects, LIR was solely a linear function of the $T_{meristem}$ in cucumber plants (in the range 18-26°C) even under large temperature differences (up to 8 °C) between the bud (approximated by $T_{meristem}$) and the rest of the plant (Chapter 3). This signifies that $T_{meristem}$ is the temperature to be related with LIR.

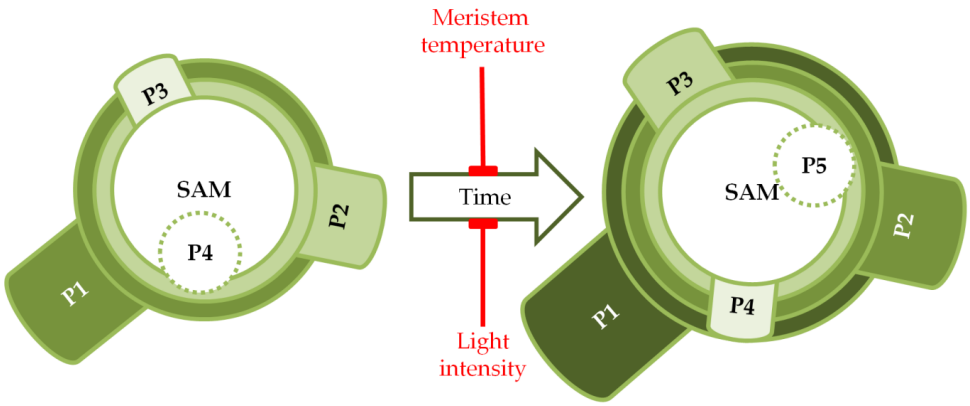

Fig. 2. The time between the initiation of two successive primordial leaves (P4 and P5) on the shoot apical meristem (SAM), decreases 1) with increasing meristem temperature within a normal temperature range (e.g. for cucumber plants 18-26 °C) and 2) with increasing light intensity until a certain low light level (e.g. for cucumber and tomato plants 6.5 mol m^{-2} d^{-1}) as indicated in this study.

$T_{meristem}$, even being a large part of the puzzle in the linkage between LIR and the aerial environment, does not fully complete the story. In this study we clearly showed that LIR substantially decreased with decreasing photosynthetic photon flux density (PPFD) in tomato and cucumber plants below a certain low light level (6.5 mol m^{-2} d^{-1}; Fig. 2, Chapter 5) when $T_{meristem}$ was kept fairly constant (Table 1, Chapter 5). LIR reduction when PPFD at shade levels was previously observed in various species (*Solanum lycopersicum* L., Hussey 1963a; *Cucumis sativus*

L., Newton 1963; *Arabidopsis thaliana* L., Chenu *et al.* 2005; Cookson *et al.* 2005; *Trifolium repens* L., Christophe *et al.* 2006). In cucumber and tomato plants, LIR showed a saturating response to PPFD (in the range 2.5 to 13.2 mol m^{-2} d^{-1}), although LIR in cucumber plants was two-fold higher than in tomato plants (Fig. 2, Chapter 5).

Relating LIR solely to T_{air} is not always valid because: 1) temperature-responses of LIR are solely attributed to $T_{meristem}$ (Chapter 3), 2) $T_{meristem}$ is not only a function of T_{air} (Fig. 1; Chapter 2), 3) LIR is not only a function of $T_{meristem}$ but also a function of PPFD (Chapter 5). In addition, the relative importance of the two different factors ($T_{meristem}$ and PPFD) with respect to LIR, changes depending on the specific environment that a plant is subjected. For instance, at high light levels, PPFD is not influential for LIR while at low light levels LIR may decrease by 40% (Chapter 5). In conclusion, the relationship between LIR and aerial environment is properly specified only when the actual influential factors, such as the $T_{meristem}$ and PPFD, are directly linked to LIR (Fig. 2).

6.1.2. The fasts and slows of leaf initiation: an (eco-) physiological approach

SAM is the fountain and simultaneously the architect of the shoot (Chapter 1 and 4). The rate in which the process of leaf/phytomer initiation is repeated in the SAM (Fig. 2) determines the number of phytomeres on the shoot over time (Chapter 3) but also regulates the distribution of leaf area and biomass per phytomer (Chapter 4).

Regarding temperature responses, the sole dependence of LIR on the apical bud temperature in indeterminate species, such as cucumber (Chapter 3), indicates that a plant can track the temperature on the top of the canopy and operate correspondingly. A higher $T_{meristem}$, within a normal growth temperature range, is not only indicating optimum growth temperatures on the top of the shoot as it can be the outcome of higher radiation (yielding higher PPFD) or lower wind speeds (Chapter 2). These environmental conditions are also beneficial for plant growth as they provide higher photosynthesis due to increased PPFD and lower chance of upper shoot damage or wilting due to high wind speeds. Consequently, when at higher $T_{meristem}$, the plant develops rapidly (i.e. rapid addition of phytomeres on the shoot) towards a more optimum environment for growth, while when at lower $T_{meristem}$, the plant develops slower towards less optimum

environments. Following this reasoning, in a plant canopy with multiple shoots the locality of temperature perception regarding LIR may yield disproportional outgrowth of shoots to serve a canopy shape which is more beneficial for plant growth when a plant is subjected to spatially diverse environments.

LIR is limited by light at low PPFDs (Chapter 5). A plant limits its investment to more phytomeres based on the photosynthate available for growth. The decrease only at very low light levels indicates that beyond a threshold light level, LIR is maintained (Chapter 5). This suggests that, with decreasing PPFD until this threshold light level, priority is given to producing new phytomeres rather than maintaining the same growth rates per organ.

The priority for producing new leaves/phytomeres is also obvious from the fact that LIR followed the apical bud temperature even when the latter increased far beyond the temperature of the rest of the plant. This resulted in more phytomeres but less leaf area and biomass per phytomer (Chapter 4).

Conclusively, changes in leaf initiation rate can be considered as important determinants of plant phenotypic plasticity to spatial and temporal variations in the aerial environment.

The mechanisms underlying leaf initiation were extensively studied from molecular to organ level (e.g. Fleming *et al.* 1997; Ha *et al.* 2010; Besnard *et al.* 2011; Sassi and Vernoux 2013). In addition, the responses of LIR to the environment were also extensively studied (e.g. Granier and Tardieu 1998; Granier *et al.* 2002; Cookson *et al.* 2005; Savvides *et al.* 2014). However, still little is known on the linkage between the mechanisms underlying leaf initiation, leaf initiation rates and the environment.

LIR linearly increased with the increase in local tissue temperature (Chapter 3) and decreased with decreasing PPFD (Chapter 5). The decrease with decreasing PPFD was at least partly related to the local photosynthate availability (Chapter 5). These suggest that LIR is influenced: 1) at organ level by temperature and 2) at plant level by PPFD (i.e. as the meristematic tissues are considered as non-autotrophic; Turgeon 1989).

The increase in LIR with increasing temperature and light intensity was related with increasing cell division rates in the SAM (Milthorpe 1959; Milthorpe and Newton 1963). The increase in cell division rates were related to shorter G1 cell-cycle phase with increasing temperature (Francis and Barlow 1988). The

relation between LIR and cell division rate in the SAM is supported by a study in which transgenic tobacco plants expressing CycD2;*At* (*A. thaliana* D2-type cyclin; Mironov *et al.* 1999) showed accelerated G1 cell-cycle phase and hence accelerated cell division and higher LIR (Cockcroft *et al.* 2000). In another study, sugar availability also influenced cell division rates by shortening G1 cell-cycle phase via controlling the expression of CycD2 and CycD3 (Riou-Khamlichi *et al.* 2000). Based on these similarities it can be argued that T_{meristem} and PPFD effects on LIR are based on a common control over the local-to-the-SAM cell division rates.

It would be rather easy to relate temperature and light intensity effects on LIR mainly to changes in meristematic cell division rates. However, the initiation of a new leaf primordium on the flanks of the SAM depends also on the outgrowth of the previously formed primordia (Reinhardt *et al.* 2003; Golz 2006). The dependence of leaf initiation on the initial expansion of the earlier formed primordial leaves suggests that the effects of temperature and light level on leaf initiation are not only a function of the local-to-the-SAM changes in cell division rate but also of the simultaneous effects on the outgrowth of the earlier formed primordial leaves. Hence, the SAM plus the earlier initiated primordial leaves (i.e. the shoot apex) should be considered as a unified system whose function is highly dependent on intrinsic interactions and extrinsic cues derived from the immediate environment or the rest of the plant.

6.1.3. Future perspectives in the study of leaf initiation process and its linkage to the aerial environment

In this study, the effects of temperature and light levels on LIR were separately investigated (Chapter 3 and 5). The presence of interactions between light and temperature signaling pathways in plant development was previously stated (Franklin 2009). Investigating for potential interactions regarding LIR in the future would be of great importance and interest.

The effects of PPFD on LIR were related to the local photosynthate availability (Chapter 5). This does not necessarily exclude the possibility that the effects of PPFD on LIR are partly mediated by local or systemic light signals. Previous research suggested that specific photoreceptors may sense low and high light intensities and mediate changes in specific plant processes (e.g. internode elongation, Ballare *et al.* 1991). In addition, the local-to-the-SAM perception of light

was already indicated (Yoshida *et al.* 2011). Spectral quality is another property of the light environment. Recent studies have revealed that light quality is influencing primordial leaf expansion (Carabelli *et al.* 2007) and that, specific photoreceptors may be involved in the leaf initiation process (Sysoeva and Markovskaya 2013). The linkage of the process of leaf initiation and light environment is an open and promising field due to the various missing links and hence the many research questions awaiting answers.

In this study, LIR was estimated as the number of leaves/phytomeres initiated per unit of time (days) based on time periods of weeks because even in fast growing species, such as tomato and cucumber, the LIR is ranging between 0.3-1.5 leaves day^{-1}. The initiation of less than two leaves per day does not allow the quantification of the diel course of leaf initiation. Consequently, the linkage of LIR to the aerial environment in a diel basis seems challenging at the moment. However, this would facilitate the investigation of the diel patterns in leaf initiation as regulated by intrinsic and extrinsic factors (like e.g. in leaves and roots; Ruts *et al.* 2012).

The relation between the rates of developmental processes, like leaf initiation (LIR), and temperature follow a well-known predictable pattern (Chapter 1 and 3). Based on this well-defined pattern, the concept of thermal time was built and used to model and predict LIR based on temperature. Despite its wide usage, thermal time does not have a concrete physiological basis (Granier *et al.* 2002). Over the last decades, progress has been made on unravelling the mechanisms underlying leaf initiation. Consequently, leaf initiation can be considered a model process in revealing the physiological basis of thermal time.

6.2. Leaf initiation and subsequent leaf expansion: two coordinated processes?

In Chapter 4, light has been shed on the substantial effects of altered apical bud temperature on plant phenotype. When T_{bud} was altered from the rest of the plant, changes in number of phytomeres were accompanied by changes in leaf area and biomass distribution per phytomer while no substantial effects on plant growth

were observed (Chapter 4). These findings indicate that T_{bud} and subsequently LIR are largely determining the distribution of leaf area and biomass along the shoot.

When T_{bud} decreased below or increased beyond T_{plant} this resulted in larger and smaller final leaf area per leaf (FLA) respectively while when T_{bud} uniformly increased or decreased with T_{plant} no differences were observed in FLA (Fig. 3, Chapter 4).

When T_{bud} did not differ from T_{plant}, LIR and average leaf expansion rate (LER) were linearly related to T_{bud} (Chapter 3) and leaf temperature during leaf expansion (T_{leaf}; Fig. 3c, Chapter 4) respectively while leaf expansion duration (LED) was negatively linearly related to T_{leaf} (Fig. 3a, Chapter 4) in agreement with previous studies (Granier and Tardieu 1998; Granier *et al.* 2002). This indicates a correlation between leaf initiation, the subsequent leaf expansion and the production of fully expanded leaves (Turc and Lecoeur 1997; Granier *et al.* 2002). This also suggests that the number of leaves expanding on the shoot is maintained with increasing plant temperature in *Cucumis sativus* plants in agreement with a study on *Pisum sativum* (Turc and Lecoeur 1997). In this study, the increase in LER fully counterbalanced the decrease in LED resulting in no changes in the FLA with increasing T_{leaf} (Table 1 and Fig. 3e, Chapter 4). Overall, with uniformly increasing plant temperature, plant leaf area increased without changes in leaf area per leaf.

With T_{bud} increasing beyond T_{plant} the increase in the number of unfolded leaves was compensated by a decrease in FLA (Fig. 3, Chapter 4). On the other hand, with decreasing T_{bud} below T_{plant} the decrease in the number of unfolded leaves was compensated by an increase in FLA. Changes in leaf area per leaf were related to alterations in LED and/or LER depending on the leaf number (Table 1, Chapter 4). LED_{dd} and/or LER_{dd} (LED and LER normalized for thermal time) linearly decreased with increasing T_{bud}-T_{leaf} resulting in reduced FLA (Table 1, Chapter 4).

Previous studies suggested that temperature-responses of leaf expansion can be solely related to T_{leaf} (Granier and Tardieu 1998; Granier *et al.* 2002). In this study we have shown that this relation stands only when plants are subjected to uniform plant temperatures (Fig. 3a, Chapter 4). The correlations between the leaf expansion determinants (normalized for T_{leaf} effects) and FLA with T_{bud}-T_{leaf} irrespective of T_{leaf} imply that leaf expansion is not a function of T_{leaf} alone but at

least a function of two different temperatures across the plant, namely T_{bud} and T_{leaf}, during leaf expansion.

T_{bud} during leaf expansion is influencing the number of phytomeres initiated and therefore the number of growing organs present on the shoot during the expansion of a certain leaf while T_{leaf} is regulating the duration of leaf expansion. The more the T_{bud} increases or decreases in relation to T_{leaf} the more the number of growing organs will increase or decrease respectively during the expansion of a certain leaf. The number of growing organs during leaf expansion was shown to affect leaf expansion and FLA (Alderfer and Eagles 1976; Marcelis 1993b). In this study, the accumulation of more, or less phytomeres over time without the respective increase or decrease in the whole plant temperature was accompanied with smaller or larger leaves respectively. This suggests that the effect of altered T_{bud} on leaf expansion and FLA is related to the number of growing phytomeres during leaf expansion. This is in agreement with the similar FLA observed with uniformly increasing plant temperature. In this case, the number of growing phytomeres during leaf expansion was maintained as suggested by the negative proportional relation between LIR (Chapter 3) and LED (Fig. 3a, Chapter 4) with increasing plant temperature.

Previous studies suggested that leaf initiation and subsequent leaf expansion are coordinated processes across temperatures even under temporal temperature fluctuations (Granier and Tardieu 1998; Granier *et al.* 2002; Parent *et al.* 2010). This coordination was based on similar correlations of LIR, LER and 1/LED with temperature (Granier *et al.* 2002). In this study, we have interfered in these correlations obtained under uniform plant temperatures by altering T_{bud} and subsequently LIR while maintaining T_{plant}. Our findings suggest that the correlation observed between leaf initiation and leaf expansion with increasing plant temperature is based on the constant number of growing phytomeres (or leaves) on the plant. Increasing or decreasing the number of growing phytomeres due to a sole increase or decrease in T_{bud} decreases or increases individual leaf expansion respectively. These findings strengthen the notion on strict coordination between different developmental processes, like leaf initiation and leaf expansion. In agreement with Granier and Tardieu (2009), our findings show that leaf expansion is determined by mechanisms at different organizational levels.

6.3. Implications in the study of plant (eco-) physiology

6.3.1. Plant temperatures and experimental practice

Tomato and especially cucumber plants show high LIR and steep responses of LIR to temperature (Chapter 3; Marcelis 1993b; Heuvelink 2005). Based on published relations between T_{air} and LIR for tomato (Heuvelink 2005) and cucumber (Marcelis 1994), a 'small' diel deviation of 1 ^{o}C from 20 ^{o}C would substantially alter the LIR by 5% in tomato and 9% in cucumber plants (Chapter 2). Likewise, substituting $T_{meristem}$ with T_{air} potentially results in overlooking the important effects of environmental variables such as wind speed, radiation, and vapour pressure deficit on $T_{meristem}$ and via that on LIR. In addition, overlooking the effects of increasing radiation levels on $T_{meristem}$ (Chapter 2) may lead to erroneous linkage between the PPFD and LIR (Chapter 5). This yields inaccurate study and/or prediction of $T_{meristem}$ and other (e.g. PPFD) effects on LIR. $T_{meristem}$ quantification is needed to properly distinguish between the factors influencing LIR through effects on $T_{meristem}$ and factors influencing LIR directly (e.g. daylength; Jamieson *et al.* 1995; e.g. radiation; Trouwborst *et al.* 2010). Therefore, $T_{meristem}$ quantification is desirable to properly study the plant-environment interactions.

Quantifying the temperature of a single plant organ along the plant does not necessarily yield a proper estimation of the whole plant temperature. For instance, vertical intra-plant temperature differences, mainly caused by vertical microclimatic differences, were observed in nature (Gibbs and Patten 1970), field crop cultivation (Gardner *et al.* 1981) and in protected cultivation (Kempkes and van de Braak 2000; Li *et al.* 2014). The existence of intra-plant temperatures differences, for example between the apical bud and the lower plant, yielded substantially different phenotypes in cucumber plants when compared to plants subjected to uniform plant temperatures (Chapter 4). The resulted changes in phenotypic traits were related either solely to apical bud temperature (e.g. LIR; Chapter 3) or the degree of the difference between the bud temperature and the temperatures of the lower plant (e.g. final leaf area; Chapter 4). Therefore, in agreement with Poorter *et al.* (2012), proper quantification of plant temperature in

time and space is necessary to avoid the misinterpretation and to enhance the quality of experimental findings.

In this study, plant temperature measurements were performed mainly by custom-made fine thermocouples (Chapter 2). After a suitable calibration (Chapter 2) these cost-efficient sensors, with proper positioning, were able to accurately measure plant temperatures along the plant (Chapters 2, 3, 4, 5). Plant temperatures can also be precisely monitored by the use of infrared imaging (Chapter 2). Although not as cost-efficient as the thermocouples, infrared cameras may be used to monitor plant temperatures in distance reducing the risk of tissue damage. In this case, recent studies on thermal imaging and plant temperature measurements are useful in addition to the manuals provided by the cameras-producing companies to precisely measure plant temperatures (e.g. Jones 2004; Costa *et al.* 2013).

6.3.2. Custom-made extension for gas exchange measurements on other-than-planar-leaf plant structures

Gas exchange measuring equipments are mainly designed and built to satisfy the need for measurements on the main photosynthesizing and transpiring plant organ, the planar leaf. This limits the usage of such important apparatuses to measure gas exchange of plant organs or structures that are not as plain as leaves, such as the apical bud. However, the use of custom-made extensions on the already commercially available gas exchange measuring equipments makes the measurements on non-planar plant structures possible. In this study, a custom-made chamber (Fig. 1, Chapter 2) was developed to be used in connection with a portable gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE, USA). The system was subjected to a series of tests: 1) to ensure the absence of gas (CO_2 and H_2O) leakage between the ambient and the chamber and 2) to compare the light environment (light intensity and spectral quality) in the chamber with the ambient (Chapter 2).This yielded the proper quantification of the transpiration rates (Fig. 7, Chapter 2) and net photosynthetic rates of the apical bud (Fig. 5, Chapter 5). Similar adjustments can be used for gas exchange measurements on different plant species and other plant organs, such as inflorescences and fruits (or fruit trusses).

6.3.3. Custom-made apparatus for organ microclimate control

Organ microclimate control was necessary in this study but also in many previous studies (e.g. Lake *et al.* 2001; Coupe *et al.* 2006; Gorsuch *et al.* 2010). This necessity arrives when aiming at unraveling 1) the local-to-the-organ effects of the environment (Chapter 3), or 2) the effects of spatial plant temperature differences on plant phenotype (e.g. Chapter 4), and/or 3) local environmental perception and long-distance signals/effects to other plant parts (e.g. Coupe *et al.* 2006).

In this study, a custom-made heating/cooling system was used to alter apical bud temperature (Fig. 1, Chapter 3). This system: successfully provided the alteration needed in apical bud temperature in comparison to the ambient air temperature, sufficiently maintained the temperature and the vapour pressure deficit in the sphere regardless the ambient conditions along the treatments that lasted almost a month (Table 2, Chapter 3) and was able to follow the apical bud during its upward movement with plant growth (Fig. 1, Chapter 3). Similar systems may be used in the future to serve the same purposes or even, with some small adjustments, for the alteration of different microclimatic variables, such as CO_2 concentration, light quality, light intensity, at organ level.

6.4. Implications in plant production systems and plant growth modeling

6.4.1. Plant temperatures and plant production systems

In open field cultivation, $T_{meristem}$ and the temperature of other plant organs strongly deviated from T_{air}, especially under extreme conditions (e.g. high radiation levels; Guilioni *et al.* 2000; Vinocur and Ritchie 2001; Guilioni and Lhomme 2006).

Even in greenhouse cultivation at high latitudes, where intensive climate control and low radiation levels occur, plant organ temperatures may substantially differ from T_{air}. Furthermore, intra-plant temperature differences (or spatial microclimate differences proposing intra-plant temperature differences) may occur depending on the climate control strategy used (Kempkes and van de Braak 2000; Trouwborst *et al.* 2010; Qian *et al.* 2012; Li *et al.* 2014).

The use of different lighting strategies may yield differences in $T_{meristem}$. For example, the use of high-pressure sodium lamps (HPS) of relatively high electrical power (600W) for top lighting yields higher $T_{meristem}$ in comparison with the use of top lighting by HPS lamps of lower el. power (400W) in combination with interlighting (light applied within canopies) by light emitting diodes (LEDs; Trouwborst *et al.* 2010). HPS lamps produce large amounts of near infrared and longwave radiation (radiative heat). The presence of a smaller or larger radiative source in the surrounding environment of the apical bud is expected to influence $T_{meristem}$ accordingly (Fig. 1).

The use of different cooling or heating strategies may yield vertical intra-plant temperature differences (Kempkes and van de Braak 2000; Qian *et al.* 2012). For instance, using a traditional heating system with overhead heating pipes (i.e. a longwave radiation source on the top of the crop) resulted in vertical plant differences, i.e. higher top and lower bottom shoot temperatures, in a chrysanthemum crop (Kempkes and van de Braak 2000). These intra-plant temperature differences were diminished by the application of a (intra-) crop heating system (Kempkes and van de Braak 2000). In another example, cooling below the tomato canopy in semi-closed greenhouses yielded vertical air temperature gradients along the shoot. Air temperature on the top of the canopy was 5 ^{o}C higher than the at the bottom of the canopy (Qian *et al.* 2012). No vertical air temperature gradients were observed when applying cooling above the tomato canopy (Qian *et al.* 2012).

The present findings (Chapter 2, 3 and 4) in combination with the knowledge derived from previous studies strongly recommend that plant temperatures instead of air temperature should be monitored or estimated in plant production systems, such as open field or protected (greenhouse) cultivation aiming a more precise prediction of plant development and crop yield. In addition, the climate control strategies used or to be used in greenhouse horticulture should be developed and tested also based on plant temperatures and not only based on microclimate variables.

Monitoring with sensors usually used in experimental practice, e.g. thermocouples, is not possible due to the extensive labor needed but also due to the challenges introduced for cultivation practices by their attachment on the plants. Thermal imaging has nowadays a plethora of applications in agriculture

(Vadivambal and Jayas 2011). Detailed monitoring of plant organ temperatures within a plant canopy seems applicable, however, only for small (experimental) agricultural plots while for larger areas of commercial cultivation a holistic monitoring is not realistic.

6.4.2. Microclimate models

The answer to the challenging, extensive and detailed monitoring of plant temperatures is the use of models able to predict organ temperatures based on microclimatic measurements (Chelle 2005). The prediction of $T_{meristem}$ based on heat exchange-related environmental variables and plant-specific traits have been successfully employed in the past in various species both in protected- (Catharanthus roseus L., Faust and Heins 1998) and open field cultivation (Zea mays L., Guilioni *et al.* 2000). In this study, the inter-specific differences observed in $T_{meristem}$ due to structural-functional differences in the apical bud (Chapter 2) suggests that for the development of such models should be taken into account: 1) the inter-specific heat exchange –related differences and 2) the developmental stage - specific differences as organ structural-functional changes are usually observed during plant development.

The intra-plant temperature differences often quantified in previous studies (Gardner *et al.* 1981; Kempkes *et al.* 2000; Li *et al.* 2014) and the important effects of altered apical bud temperature on plant phenotype observed in this study (Chapter 4) indicate the necessity for integrating organ-specific microclimate models to plant level. The prediction of whole plant temperature based on models seems challenging though not impossible. As suggested by Chelle (2005), coupling organ microclimate models with functional-structural plant models (FSPM) seems to be promising in determining the environmental perceptions by each plant organ within a plant canopy.

6.4.3 Plant growth models

Plant growth models are usually estimating the progression in plant development based on air temperature-based thermal time. The necessity to incorporate plant organ temperatures in such kind of models was previously suggested. The necessity of incorporating other-than-temperature influential factors is indicated by the important decrease in LIR at low PPFD (Chapter 5). PPFDs below 6.5 mol m-

^{2} d^{-1} can be found in protected cultivation (especially during winter at high latitudes; Marcelis and Gijzen 1998). Alteration of the relationship between development (e.g. LIR) and temperature by other environmental factors indicates the necessity of extending the development rate concept (Campbell and Norman 1998).

Coupling fundamental processes, like leaf initiation and leaf expansion, with the temperatures actually perceived by the apical bud or the leaf respectively in plant growth models is necessary (Chapter 3) but not enough. This study shows that plants are not just the sum of modules that are independently contributing to plant growth based on the local environmental perception but a sum of interconnected and highly interacting modules able to shape plant phenotype to satisfy plant needs even under spatial plant temperature differences. This knowledge can be integrated in plant growth modeling by linking organ microclimate models (Chelle 2005) with functional structural plant models (Vos *et al.* 2010) using a systems biology approach (Baldazzi *et al.* 2012; Poorter *et al.* 2013).

Conclusions

At the outset, the findings of this thesis demonstrate that in the relation between LIR and the environment, air temperature is not the whole story. The first reason is that LIR is a function of meristem temperature and meristem temperature may largely deviate from air temperature. This deviation depends on other environmental factors and species-specific heat exchange-related traits. The second reason is that LIR is also determined by light intensity at low light levels. Therefore, the relationship between LIR and aerial environment is properly specified only when the actual influential factors, such as the T_{meristem} and PPFD, are directly linked to LIR.

Furthermore, the effects of the temperature differences between the apical bud and the rest of the plant on plant phenotype, revealed in this study, provide new insights on the importance of LIR for plant phenotypic plasticity and the coordination between leaf initiation and leaf expansion.

The findings and the methodology used in this thesis have important implications in the study of plant (eco-) physiology, plant production systems and plant growth modelling and provide new research questions for future research in this field.

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Summary

Leaf initiation rate (LIR; number of leaves initiated per day) is a widely-used measure of the number of leaves as well as the number of phytomeres initiated over time by the shoot apical meristem (SAM). Hence, LIR is a critical feature for plant architecture, plant leaf area, and therefore plant growth. It is commonly assumed that LIR is a function of shoot apical meristem temperature ($T_{meristem}$) which can be well approximated by air temperature (T_{air}). It can be argued, that relating LIR solely to air temperature may lead to substantial misapprehension of the effects of the different components of the aerial environment on LIR. These components may influence $T_{meristem}$ (e.g. solar radiation) and therefore LIR and 2) affect LIR independent of $T_{meristem}$ (e.g. photosynthetic photon flux density; PPFD). Hence, the central aim of this thesis is to more accurately link LIR to the aerial environment.

This study focuses on 1) unravelling the contribution of the different aerial environmental variables as well as the contribution of apical bud (i.e. the foliar structure in which SAM is enclosed) heat-exchange-related traits on $T_{meristem}$, 2) revealing whether the apical bud is the predominant site of temperature perception regarding LIR even under intra-plant temperature differences between the apical bud and the rest of the plant 3) determining the effects of the intra-plant temperature differences between the apical bud and the rest of the plant on plant phenotype 4) Unravelling the relation between LIR and PPFD as well as the possible relation between the potential effects of PPFD on LIR and carbon availability.

Chapter 2 focuses on unravelling the contribution of the different aerial environmental variables as well as the contribution of apical bud heat-exchange-related traits on $T_{meristem}$. The temperature of a plant organ is the net outcome of the heat exchange between the organ and its environment. Besides T_{air}, other environmental variables like radiation, wind speed, and vapour pressure deficit are strongly involved in the heat exchange processes between plant organs and

their environment. The enclosure of the SAM within the bud suggests that meristem microenvironment and therefore $T_{meristem}$ are strongly related to the bud structure and function. The type, number, size, shape and arrangement of the organs comprising the bud vary enormously between species. Therefore, $T_{meristem}$ may deviate from T_{air} in a species-specific way. Environmental variables (air temperature, vapour pressure deficit, radiation, and wind speed) were systematically varied to quantify the response of $T_{meristem}$. This response was related to observations of bud structure and transpiration. Tomato and cucumber plants were used as model plants since their apical buds are morphologically distinct and they are usually growing in similar environments. $T_{meristem}$ substantially deviated from T_{air} in a species-specific manner under moderate environments. This deviation ranged between -2.6 and 3.8 ^{o}C in tomato and between -4.1 and 3.0 ^{o}C in cucumber. The lower $T_{meristem}$ observed in cucumber was linked with the higher transpiration of the bud foliage sheltering the meristem when compared with tomato plants. We here indicate that for properly linking growth and development of plants to temperature in future applications, for instance in plant growth models, $T_{meristem}$ should be quantified or estimated instead of T_{air}, as a species-specific trait highly reliant on various environmental factors.

Chapter 3 focuses on revealing whether the apical bud is the sole site of temperature perception regarding LIR even under intra-plant temperature differences between the apical bud and the rest of the plant. In most models, relating plant development to the environment, not the temperature of the SAM, but the ambient- (T_{air}) or a general plant-temperature is used to calculate LIR. In many natural and agricultural environments, the temperature of the apical bud (T_{bud}) may significantly differ from T_{air} or the temperature of the rest of the plant (T_{plant}). If T_{bud} solely influences LIR, its poor approximation will lead to serious misinterpretation of experimental results and miscalculations in models. If beside T_{bud}, T_{plant} also influences LIR (through systemic signals), predictions will become even more problematical. We investigated whether LIR solely depends on T_{bud} when T_{bud} is independently altered from T_{plant}. Using a custom-made device, T_{bud} was altered in cucumber plants yielding 9 combinations of T_{bud}/T_{plant} between 18-26 ^{o}C and LIR was quantified. LIR increased by 12% per ^{o}C of T_{bud} regardless T_{plant}. The sole temperature-response of LIR to T_{bud}, even under major intra-plant temperature differences, implies a strong and singular relation between bud

function and local temperature perception. Consequently, accurate measurements or realistic estimates of T_{bud} should be used in experimental and modelling studies in which plant development is a key issue.

Chapter 4 shows the effects of the intra-plant temperature differences between the apical bud and the rest of the plant on plant phenotype. Leaf initiation and subsequent leaf expansion were suggested to be well-correlated processes with increasing plant temperature resulting in well-defined plant phenotypes. In chapter 3 it was shown that T_{bud} influences leaf initiation independent of T_{plant}. Though, the effects of altered T_{bud} on leaf expansion and whole plant phenotype remained to be unravelled. Increasing T_{bud} beyond T_{plant} resulted in more and smaller leaves while decreasing T_{bud} below T_{plant} resulted in less and larger leaves. This offset between leaf number and individual leaf area indicates a strict systemic coordination between leaf initiation and leaf expansion. The same patterns as leaf area distribution were observed for biomass distribution across phytomeres. Cucumber plants adjust their phenotype to increased or decreased T_{bud} by reallocating their investments into more or less phytomeres respectively.

Chapter 5 focuses on unravelling the relation between LIR and PPFD as well as the possible relation between the potential effects of PPFD on LIR and carbon availability. Radiation substantially affects leaf initiation rate (LIR), a key variable for plant growth, by influencing the heat budget and therefore the temperature of the shoot apical meristem (chapter 2). The photosynthetically active component of solar radiation (photosynthetic photon flux density; PPFD) is critical for plant growth and when at shade to moderate levels may also influence LIR via limited photosynthate availability. Cucumber and tomato plants were subjected to different PPFDs (2.5–13.2 mol m^{-2} d^{-1}) and then LIR, carbohydrate content and diel net CO_2 uptake of the apical bud were quantified. LIR showed saturating response to increasing PPFD in both species. In this PPFD range, LIR was reduced by 20% in cucumber and by 40% in tomato plants. Carbohydrate content and dark respiration were substantially reduced at low PPFD. LIR may be considered as an adaptive trait of plants to low light levels, which is likely to be determined by the local photosynthate availability. In tomato and cucumber plants, LIR can be markedly reduced at low PPFD in plant production systems at high latitudes, suggesting that models solely based on thermal time may not precisely predict LIR at low PPFD.

Chapter 6, the general discussion, brings together the findings described in Chapters 2 to 5 to give 1) a holistic answer to the question 'why air temperature is not the whole story when linking leaf initiation to the aerial environment', 2) to discuss the implications in the study of plant (eco-) physiology, plant production systems and plant growth modelling, 3) to discuss future perspectives in the study of leaf initiation in response to the environment and 4) to initiate the critical matter of plant temperature heterogeneities and their impacts on plant phenotype.

Samenvatting

De bladafsplitsingsnelheid (LIR; in deze samenvatting worden Engelse afkortingen gebruikt) is een veelgebruikte maat voor de snelheid waarmee nieuwe bladeren en fytomeren worden aangelegd door het topmeristeem van een scheut (SAM). De LIR beïnvloedt in belangrijke mate de architectuur, het bladoppervlak en daarmee ook de groei van een plant. In het algemeen wordt aangenomen dat de LIR sterk wordt beïnvloed door de temperatuur van de SAM ($T_{meristem}$) en vaak word eveneens aangenomen dat de temperatuur van de omringende lucht (T_{air}) een goede benadering is voor $T_{meristem}$. Deze aanname kan echter leiden tot aanzienlijke misvattingen over de effecten van omgevingsfactoren op de LIR. Zo is het in principe mogelijk dat omgevingsfactoren zoals luchtvochtigheid of straling, naast hun eigen specifieke effecten op de LIR, de LIR ook nog indirect beïnvloeden via $T_{meristem}$. De straling van de zon, bijvoorbeeld zou de LIR kunnen beïnvloeden via de component fotosynthetisch actieve straling (PPFD) maar ook via het effect van de component warmtestraling op de temperatuur van het topmeristeem ($T_{meristem}$). Dit proefschrift heeft als doel te komen tot een nauwkeurigere omschrijving van de relatie tussen de LIR en het bovengrondse klimaat.

Dit onderzoek richt zich daarom op 1) het ontrafelen van de bijdragen van verschillende bovengrondse omgevingsfactoren en enkele functionele en structurele eigenschappen van de eindknop, welke bestaat uit het topmeristeem en enkele nog niet ontvouwde bladeren, op $T_{meristem}$, 2) het ophelderen of $T_{meristem}$ de enige bepalende temperatuur is in relatie tot LIR wanneer de temperatuur van het topmeristeem verschilt van de temperatuur van de rest van de plant, 3) het bepalen van effecten van temperatuurverschillen tussen de eindknop en de rest van de plant op het fenotype van de plant en 4) het ontrafelen van de relatie tussen LIR en fotosynthetisch actieve straling (PPFD) en de mogelijke rol van assimilaten beschikbaarheid daarbij.

Nadat in **Hoofdstuk 1** de achtergronden en doelstelling van het onderzoek uiteen zijn gezet, richt **Hoofdstuk 2** zich op het ontrafelen van de bijdrage van

verschillende bovengrondse omgevingsfactoren en enkele eigenschappen van de eindknop op de warmtebalans en de temperatuur van de eindknop en het topmeristeem ($T_{meristem}$). De temperatuur van een plantenorgaan wordt beïnvloed door het netto warmtetransport tussen het orgaan en zijn omgeving. Behalve de temperatuur van de omringende lucht (T_{air}) zijn ook straling, windsnelheid, en luchtvochtigheid van grote invloed op het warmtetransport tussen plantenorganen en hun omgeving. Het insluiten van het topmeristeem in een eindknop doet vermoeden dat het microklimaat om het topmeristeem en dus ook $T_{meristem}$ sterk gerelateerd zijn aan de structuur en functie van de knop. Het type, aantal, grootte, vorm en rangschikking van de organen die samen de knop vormen verschillen sterk tussen plantensoorten. Daarom is het aannemelijk dat eventueel optredende verschillen tussen $T_{meristem}$ en T_{air} ook soortafhankelijk zijn. Omgevingsvariabelen (luchttemperatuur en –vochtigheid, straling, en windsnelheid) werden systematisch gevarieerd om de reactie van $T_{meristem}$ te kwantificeren. Deze respons werd gerelateerd aan de morfologische structuur en transpiratie van de eindknop. Tomaten- en komkommerplanten werden gebruikt als modelplanten omdat hun eindknoppen morfologisch verschillend zijn en ze vaak geteeld worden in vergelijkbare omgevingen. $T_{meristem}$ week vaak aanzienlijk af van T_{air} en er waren duidelijke verschillen tussen beide soorten. De afwijkingen tussen $T_{meristem}$ en T_{air} liepen uiteen van -2.6 tot 3.8 ^{o}C in tomaat en tussen -4.1 en 3.0 ^{o}C in komkommer. De lagere $T_{meristem}$ in komkommer ten opzichte van tomaat kwam door de hogere transpiratie van alle nog niet ontvouwde bladeren die het topmeristeem bij komkommer omsloten. Deze resultaten laten zien dat voor een goede koppeling van groei en ontwikkeling van planten aan temperatuur, zoals bijvoorbeeld in gewasgroeimodellen, de gemeten of geschatte $T_{meristem}$ gebruikt zou moeten worden plaats van T_{air}. Daarbij is de relatie tussen bovengrondse klimaatfactoren en $T_{meristem}$ soort specifiek.

Hoofdstuk 3 richt zicht op de vraag of de bovengrondse eindknop de enige plaats is waar temperatuur waargenomen wordt in relatie tot de LIR, ook wanneer er aanzienlijke temperatuurverschillen bestaan tussen de eindknop en de rest van de plant. In de meeste modellen die plantontwikkeling relateren aan de omgeving wordt niet de temperatuur van de SAM, maar de omgevingstemperatuur (T_{air}) of een algemene planttemperatuur gebruikt om de LIR te berekenen. In de natuur maar ook in agrarische productie systemen, kan de

temperatuur van de eindknop (T_{bud}) aanzienlijk verschillen van de temperatuur van de lucht T_{air} en de temperatuur van de rest van de plant (T_{plant}). Wanneer voor de LIR eigenlijk alleen T_{bud} van belang is zal een slechte schatting van de T_{bud} leiden tot foutieve interpretaties van proefresultaten en afwijkingen in modellen. Deze problemen worden nog groter als LIR niet alleen door T_{bud} maar ook door T_{plant} wordt beïnvloed (via systemische signalen). We hebben onderzocht of de LIR alleen afhankelijk is van de T_{bud} door T_{bud} onafhankelijk van T_{plant} te veranderen. Met behulp van een speciaal voor dit onderzoek gemaakt microklimaat regelsysteem en een transparante omhulling om de eindknop, werd T_{bud} in komkommerplanten onafhankelijk van T_{plant} gevarieerd. Dit resulteerde in 9 combinaties van T_{bud} en T_{plant} tussen de 18-26 ^{o}C. Daarbij werd de LIR gemeten. De LIR nam toe met 12% per ^{o}C T_{bud}, onafhankelijk van T_{plant}. Het feit dat de LIR alleen reageerde op de T_{bud}, zelfs bij aanzienlijke temperatuurverschillen binnen de plant, veronderstelt een sterke en unieke relatie tussen het functioneren van de eindknop en de lokale temperatuur. Daarom zouden nauwkeurige metingen of realistische schattingen van T_{bud} moeten worden gebruikt in experimenten en modelstudies waarin de plantontwikkeling centraal staat.

Hoofdstuk 4 laat de effecten van temperatuurverschillen tussen de bovengrondse eindknop en de rest van de plant op het fenotype van de plant zien. De resultaten suggereren dat bladaanleg en de daaropvolgende strekking sterk gecorreleerde processen zijn, waarbij een toename in planttemperatuur resulteert in duidelijk gedefinieerde fenotypes. In hoofdstuk 3 werd al aangetoond dat T_{bud} de bladaanleg beïnvloedt onafhankelijk van T_{plant}. Echter, de effecten van de gewijzigde T_{bud} op bladstrekking en op het fenotype van de plant zijn daarmee nog niet duidelijk. Als de T_{bud} hoger wordt dan de T_{plant} leidt dit tot meer en kleinere bladeren, terwijl een lagere T_{bud} dan T_{plant} leidt tot minder en grotere bladeren. Deze balans tussen het aantal bladeren en het bladoppervlakte per blad wijst op een strikte systemische coördinatie tussen bladaanleg en bladstrekking. De verdeling van biomassa over de fytomeren van de plant vertoonde hetzelfde patroon. Komkommerplanten passen hun fenotype aan een toe- of afname van T_{bud} aan, door hun investeringen te verdelen over respectievelijk meer of minder fytomeren.

Hoofdstuk 5 richt zich op het ontrafelen van de relatie tussen LIR en PPFD en op de relatie tussen de potentiële effecten van PPFD op LIR en de beschikbaarheid

van assimilaten. Straling, een belangrijke omgevingsfactor voor de groei van planten, heeft een sterk effect op de LIR door de warmtebalans en de temperatuur van het topmeristeem ($T_{meristem}$) van de scheut te beïnvloeden (hoofdstuk 2). De PPFD is van cruciaal belang voor de groei van planten. Bij weinig licht kan de PPFD ook de LIR beïnvloeden door een beperking van de beschikbaarheid van koolhydraten voor groei. Komkommer- en tomatenplanten werden geteeld bij verschillende PPFDs (2.5 – 13.2 mol m^{-2} d^{-1}) en de LIR, het koolhydratengehalte en de dagelijkse netto CO_2-opname van de eindknop werden gemeten. In beide soorten vertoonde de LIR een verzadigende reactie op een toename van de PPFD. In deze range van PPFD nam de LIR af met 20% in komkommer en met 40% in tomatenplanten. Het gehalte aan koolhydraten en de donkerademhaling namen beide sterk af bij lage PPFD. De LIR kan worden beschouwd als een adaptieve eigenschap van planten aan lage lichtniveaus, wat waarschijnlijk wordt bepaald door de lokale beschikbaarheid van koolhydraten. Tomaten en komkommers worden op grote schaal gekweekt in kassen, gelokaliseerd op relatief hoge breedtegraden en dus vaak onder lage PPFD niveaus. De sterk afnemende LIR onder lage PPFDs doet vermoeden dat gewasgroeimodellen, die de LIR bereken op basis van uitsluitend de thermische tijd, LIR niet correct voorspellen bij lage PPFD.

Hoofdstuk 6, de algemene discussie, brengt de bevindingen die beschreven zijn in hoofdstukken 2 tot 5 samen om 1) een holistisch antwoord te geven op de vraag 'waarom luchttemperatuur niet 'het hele verhaal' is in het koppelen van bladaanleg aan de omgeving, 2) om het belang van deze bevindingen voor de plant(eco)fysiologie en toepassingen in plantproductiesystemen en gewasgroeimodellering te bespreken, 3) om de toekomstige onderzoeksmogelijkheden met betrekking tot bladaanleg in reactie op de omgeving te bespreken, en 4) om de heterogeniteit in planttemperaturen en hun effecten op fenotype te bespreken.

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Curriculum Vitae

Andreas Savvides was born on January 17th 1983 in Lemesos, Cyprus. He attended Paralimni High School where he obtained a High School diploma in 2000. After a two-year military service in Cyprus he undertook his higher education studies in School of Agricultural Sciences at Aristoteleion University of Thessaloniki in Greece. For his BSc thesis he worked in Agricultural research institute of Cyprus and he investigated the appearance of tomato viruses in Cyprus. After obtaining his BSc (5-year) degree (Agronomy/Plant protection) in 2007 he continued his studies in Wageningen University & Research Centre, the Netherlands. For his MSc thesis he investigated the impacts of light quality on leaf hydraulic architecture, sensitivity to water stress and photosynthesis. His MSc thesis resulted in publication in *Journal of Experimental Botany*. At the end of 2009 he finalized his MSc studies (Plant Sciences/Greenhouse Horticulture) and started his PhD research in Horticulture & Product Physiology group (former Horticultural Supply Chains group) and UR Greenhouse Horticulture in Wageningen University & Research Centre. This thesis is the outcome of his PhD research.

List of publications

Papers published in refereed journals

Savvides A, Ntagkas N, van Ieperen W, Dieleman JA, Marcelis LFM. 2014. Impact of light on leaf initiation: a matter of photosynthate availability in the apical bud? *Functional Plant Biology*, 41: 547-556.

Savvides A, van Ieperen W, Dieleman JA, Marcelis LFM. 2013. Meristem temperature substantially deviates from air temperature even in moderate environments: is the magnitude of this deviation species-specific? *Plant, Cell & Environment*, 36: 1950-1960.

Fanourakis D, Pieruschka R, **Savvides A**, Macnish AJ, Sarlikioti V, Woltering, EJ. 2013. Sources of vase life variation in cut roses: a review. *Postharvest biology and Technology* 78: 1-15.

Savvides A, Fanourakis D, van Ieperen W. 2012. Co-ordination of hydraulic and stomatal conductances across light qualities in cucumber leaves. *Journal of Experimental Botany* 63(3): 1135-1143.

Papers to be published in refereed journals

Savvides A, Dieleman JA, van Ieperen W, Marcelis LFM. Leaf initiation is solely dependent on the apical bud temperature even under large bud-plant temperature differences (submitted).

Savvides A, van Ieperen W, Dieleman JA, Marcelis LFM. Phenotypic plasticity to altered apical bud temperature: more leaves smaller leaves and vice-versa (to be submitted).

Conference proceedings

Van Ieperen W, **Savvides A**, Fanourakis D. 2012. Red and blue light effects during growth on hydraulic and stomatal conductance in leaves of young cucumber plants. *Acta Horticulturae (ISHS)* 956: 223-230.

Other (professional) publications

Dieleman JA, De Gelder A, Janse J, Lagas P, Eveleens-Clark BA, Qian T, Elings A, Steenhuizen JW, Stanghellini C, Nederhoff EM, **Savvides A**, Farneti B, De Visser R, Woltering EJ, Marcelis LFM (2012). Verticale temperatuurgradiënten in geconditioneerde kassen: Effecten op groei, ontwikkeling en onderliggende processen bij tomaat. Wageningen : Wageningen UR Glastuinbouw, (Rapporten GTB 1122) - p. 142.

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Abstracts

Savvides A, Ntagkas N, Van Ieperen W, Dieleman JA, Marcelis LFM. (2012). Impact of light intensity on leaf initiation in young cucumber and tomato plants: a matter of photosynthates availability? In: *Proceedings of the 7th International Symposium on Light in Horticultural Systems (Book of Abstracts)*. Leuven: ISHS, - p. 146.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

- Growth and development in relation to shoot apex temperature in greenhouse crops

Writing of project proposal (4.5 ECTS)

- Growth and development in relation to shoot apex temperature in greenhouse crops

Post-graduate courses (3.6 ECTS)

- Meta-analysis; PE&RC (2012)
- The art of crop modelling; PE&RC (2013)
- Kick start R; NCSB: Netherlands Consortium for Systems Biology (2013)
- Environmental signaling; Utrecht Summer School, Utrecht University (2013)

Laboratory training and working visits (0.3 ECTS)

- Share findings and possibilities for collaboration; Jülich Forschungszentrum, Germany (2012)

Invited review of (unpublished) journal manuscript (2 ECTS)

- Experimental and Environmental Botany: plant water relations and development (2011)
- HortScience: shoot-tip temperature and growing strategies (2011)
- Scientia Horticulturae: light effects on plant growth and leaf anatomy (2012)

Deficiency, refresh, brush-up courses (1.5 ECTS)

- Basic statistics; PE&RC (2010)

Competence strengthening / skills courses (3 ECTS)

- PhD Competence assessment; WGS (2010)
- Teaching and supervising thesis students; DO, Educational Staff development Group (2010)
- Project and time management; WGS (2011)
- Interpersonal communication; WGS (2011)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.1 ECTS)

- PE&RC Day (2010-2013)
- PE&RC Weekend (2010)

Discussion groups / local seminars / other scientific meetings (6.3 ECTS)

- FLOP: Frontier Literature in Plant Physiology (2010-2013)
- Mini seminar: the role of hormone signalling in shoot apical meristem function (2010)
- Mini seminar: environmental physiology of herbaceous horticultural crops (2010)
- How to write a world-class paper; Wageningen UR (2013)

International symposia, workshops and conferences (5.4 ECTS)

- ISHS Light Symposium; poster and oral presentation; Wageningen, the Netherlands (2012)
- 7th EPSO Conference; Poster presentation; Porto Heli, Greece (2013)

Lecturing / supervision of practical's / tutorials (3 ECTS)

- Concepts in environmental plant physiology (2010-2013)

Supervision of 3 MSc students

- How is shoot apex temperature relying on ambient environment? Interspecific variation and comparisons with other plant organs
- Leaf initiation rate responds to irradiance: a matter of photosynthesis availability to the apical bud
- Light quality and effect on leaf initiation rates

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